# HIGHLIGHTS OF PROGRESS IN RESEARCH

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HIGHLIGHTS OF PROGRESS IN RESEARCH
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## VIRUS DISEASES AND VACCINES

Research conducted and supported by the National Institutes of Health during the year 1971 included many studies directed at improving and developing vaccines for viral diseases and related areas of prevention and treatment.

### HEPATITIS

Hepatitis constitutes a serious risk in the administration of blood and blood products. Transfused blood is known to cause more than 30,000 cases of overt hepatitis resulting in 1,500 to 3,000 deaths every year in the U.S. Since there are many subclinical cases of viral hepatitis, the actual incidence has been estimated to be as high as 150,000 cases annually.

Because of this risk, and the hope for development of a vaccine against the serum hepatitis virus, the Division of Biologics Standards (DBS) has for many years maintained an exploratory hepatitis research program. In 1968 the program was expanded under the direction of Dr. Lewellys F. Barker in order to capitalize on the finding that a hitherto unknown factor, originally found in the blood of an Australian aborigine, was intimately linked to serum hepatitis. This factor, discovered in 1965 by Dr. Baruch S. Blumberg, a former NIH investigator, behaves like an antigen when the serum of a multiple-transfused patient is used as an antiserum. The detection of Australia (Au) antigen, or hepatitis associated antigen (HAA), made it possible to identify for the first time by a laboratory method, blood that carries a high risk of transmitting post-transfusion hepatitis.

Last year National Institute of Allergy and Infectious Diseases (NIAID) and other NIH scientists developed an extremely sensitive test for hepatitis-associated antigen. Using this test, they found exposure to HAA -- as indicated by the presence of antibody -- was widespread. Many persons with no history of hepatitis, transfusions, or drug addiction had relatively high levels of antibody, suggesting to the investigators that (serum) hepatitis B may be endemic and frequently transmitted by means unrelated to injections or transfusions.

Several teams of investigators, using reagents from NIH and elsewhere, have obtained evidence that HAA has at least three subtypes, or antigenic specificities. Drs. Chung Yong Kim and Jeremiah G. Tilles, Harvard University (partially supported by NIAID, the National Institute of Arthritis and Metabolic Diseases (NIAMD), and the Division of Research Resources (DRR) used two different

laboratory techniques to identify varieties of HAA in clinical specimens. Experts feel there is a clear need to understand the distribution of these antigenic specificities within different populations and their possible correlation with host attributes and clinical conditions.

A DBS contract supports studies of methods for large-scale purification and quantitation of the antigenic subtypes to facilitate their immunologic characterization and evaluation of their significance. Progress is being made with chimpanzees as an animal model in order to gain a better understanding of serum hepatitis and ways that it may be prevented or treated.

An NIAID special fellow, Dr. Robert Ward, and his associates in Santiago, Chile, have gathered substantial evidence that transfusion of blood preincubated with modified gamma globulin reduces the incidence and severity of hepatitis. In their study, the scientists found that 5 of 1,970 patients receiving this preparation and 18 of 2,019 controls receiving plain blood, developed hepatitis. No severe cases were observed in those receiving gamma globulin, but 6 severe and 2 fatal cases occurred in the group receiving plain, whole blood.

The DBS has supported an epidemiologic study of hepatitis-associated antigen in blood donors and recipients since 1969 in New Jersey. Over 350,000 units of blood have been tested by several techniques.

The risk of hepatitis to recipients of blood from commercial donors has been shown to be three times the risk associated with voluntary donor blood. The hepatitis-associated antigen was found 6.4 times as often in blood from commercial donors. It is anticipated that this study will provide a foundation for evaluation of future antigen tests, as well as for other methods for reducing the risks of post-transfusion hepatitis.

## FEDERAL STANDARDS

The promise of significantly decreasing the incidence of post-transfusion hepatitis by identifying and eliminating blood which is positive for Australia antigen became a reality this year with the issuance of federal standards for Hepatitis-Associated Antibody (Anti-Australia Antigen).

Although limited sensitivity of available testing methods makes it impossible to detect hepatitis-associated antigen in all blood and blood products, it is estimated that 25 percent of infectious blood can now be identified and excluded from medical use.

Federal standards for Hepatitis Associated Antibody (Anti-Australia Antigen), designed to ensure the safety, purity, and potency of this biological product, were formulated by the DBS, and promulgated in final form on January 30, 1971.

The standards prescribe that to be satisfactory for release,

each filling of hepatitis associated antibody shall be tested against the Reference Hepatitis Associated Antigen (Australia Antigen) Panel and shall be sufficiently potent to be able to detect the antigen in the appropriate sera of the Reference Panel by all test methods recommended by the manufacturer. The Panel, consisting of 60 sera, is prepared for and distributed to manufacturers by the DBS.

Hepatitis associated antibody is now commercially available from six federally licensed manufacturers; additional manufacturers will undoubtedly be licensed in the coming months. At present, the manufacturers are licensed to sell antiserum from human, guinea pig, horse, and rabbit sources for use in testing. Antiserum from other sources and additional test methods are being investigated.

Last year, the DBS initiated steps to require testing by all licensed blood banks of blood, plasma, or serum for presence of hepatitis associated antigen, and elimination as donors of persons whose blood tests positive for the antigen. Final federal regulations implementing this proposal are expected to be published early in 1972.

## INFLUENZA VACCINE STUDIES

One of the basic problems in the standardization of inactivated influenca virus vaccines containing more than one strain has been the determination of the relative potency of the individual strain components. The chick cell agglutination (CCA) test was standardized in 1968 to measure reliably the total hemagglutinin content of these vaccines. Recently, Dr. Martin G. Myers and co-workers, DBS, have been able to measure each strain component of the polyvalent influenza vaccines by immunodiffusion tests such as are commonly used in immunology. Application of these techniques should allow better assessment of antigenic potency of the individual strain components of influenza vaccine.

Dr. Nicola M. Tauraso and co-workers, DBS, have also attempted to evaluate influenza vaccines produced by different processes. Considerable variation in reactogenicity was found in vaccines produced by similar methods and even among vaccine lots prepared by the same production method. The incidence of reactions was lowest among persons receiving the more highly purified vaccines. Serological studies of these vaccines revealed that more highly purified influenza virus vaccines induced antibody responses equivalent to the non-purified vaccine preparations. All U. S. manufacturers of influenza vaccine are now producing influenza vaccines purified by one of three processes: zonal ultra-centrifugation, lipid-solvent degradation, or glass chromatography. These highly purified vaccines cause significantly fewer side reactions.

## RESPIRATORY DISEASES

In an effort to control other viral respiratory diseases, the NIAID is continuing a vigorous vaccine development program. Institute scientists, and others, have been investigating the use of temperature-sensitive (ts) strains of virus for use as vaccines. These mutants are able to grow approximately as well as the wild-type virus at certain temperatures but are partially or unable to replicate at other temperatures at which wild-type virus grows normally. Armed with this knowledge, scientists are seeking mutants which will multiply to a limited extent or not at all in the lungs but will grow well in the cooler nasal passages, producing an effective immune response without pulmonary complications.

At NIAID encouraging progress is being made with ts mutants of influenza virus and respiratory syncytial (RS) virus. The latter is an important cause of serious respiratory disease during infancy and early childhood.

Two ts mutants of influenza virus $A_2/1965$ have been isolated and characterized by Dr. Robert M. Chanock and his associates. The scientists have found a high degree of correlation between growth restriction by temperature in tissue culture and in the lungs of hamsters. Infection with either mutant was found to protect the animals against subsequent exposure to wild-type virus. The behavior of these influenza ts mutants in the human host remains to be determined but it will be helpful if, in man, as in laboratory animals, the degree of attenuation correlates with laboratory experimental growth characteristics.

The RS work is a little further along. One mutant -- known as ts-1 -- which seems to have properties most favorable for potential use in a live vaccine, is being evaluated.

## MEASLES AND RUBELLA VACCINES

In contrast to respiratory virus vaccines where development work is still very much in progress, measles and rubella vaccines are already stars of a success story. In reviewing the status of measles and rubella immunization, New York University's Dr. Saul Krugman, whose work is supported by NIAID, reported that use of more than 40 million doses of measles vaccine since 1963 resulted in a 95% drop in disease incidence by 1968-69. (The rising incidence of measles in 1970-71 is the result of failure to immunize children in some poverty areas.) Dr. Krugman also found that preliminary data on the new rubella vaccines indicate that they are safe, immunogenic, and well-tolerated. Use of these vaccines should have a major impact on control of rubella which, in the epidemic year of 1964, resulted in the death or disablement of many thousand newborns whose mothers contracted the disease during pregnancy.

Since the most important persons to protect against rubella are susceptible women of childbearing age, ways are being sought to

extend vaccine use in adolescent girls and young women without running the risk of infecting a fetus. NIAID contractors have studied the feasibility of vaccinating susceptible women immediately after childbirth since conception in the 6 to 8 weeks following delivery is rare. Dr. Dorothy M. Horstmann at Yale University and her co-workers found that nearly 14% of over 1400 obstetrical patients were susceptible to rubella. Vaccination of these women 2-4 days after delivery produced high levels of antibody and no serious side-effects. The investigators suggest, in light of these findings, that rubella vaccine administered in the immediate post-partum period might be made a routine part of obstetrical care.

## COMBINED VACCINES

Research on biological products is necessarily related to current public health and medical practice. With the proliferation of new viral vaccines in recent years, there has been an increasing interest in the use of multiple vaccines combined in one injection. DBS investigators have been active over the past several years in investigating the possibility of combining certain live virus vaccines. In 1967 a combined measles-smallpox vaccine, the first commercial combination of injectable live virus vaccines, was licensed.

During the past 15 months, following extensive laboratory and clinical testing, three additional combination live virus vaccines -- measles-rubella, measles-mumps-rubella, and mumps-rubella vaccines-- have been licensed for commercial use. Safety and efficacy data on these three preparations showed 1) no increase in untoward reactions with the combined agents, as compared with each component when administered separately; and 2) no suppression of immunogenicity by one component virus on another.

Drs. Harry M. Meyer, Jr., and Paul D. Parkman, DBS, together with Dr. John Witte and other investigators from the Center for Disease Control (CDC), Atlanta, Georgia, were active in evaluating the combination vaccines during the experimental phase. Joint DBS-CDC clinical trials on the Pacific Islands of Tol and Guam provided important data relative to safety and efficacy that confirmed the earlier experience of the manufacturer.

It is anticipated that the successful combination of these and other vaccine viruses will greatly simplify immunization schedules for children, as well as minimize the required number of contacts between patient and physician.

In addition to their work on multiple antigens, Drs. Meyer and Parkman have directed studies in the past year concerning inadvertent rubella virus vaccination during pregnancy in an effort to obtain definitive information on the teratogenic potential of rubella vaccines. Data collected thus far indicate that rubella immunization can lead to persistent infection in the fetus; however, a teratogenic potential of the attenuated vaccine virus

has not yet been established. Much more information must be collected before conclusions can be reached regarding the relative risks of vaccination as compared to natural rubella during pregnancy. At present, these investigators stress the need for caution in vaccinating post-pubertal women.

## RABIES VACCINE

A good example of how results of basic studies can affect clinical management of disease was provided recently by investigators supported by the U.S.-Japan Cooperative Medical Science Program which is administered by NIAID. Research Career Awardee, Dr. Richard E. Dierks, Iowa State University, Ames, and co-workers at the Center for Disease Control, Atlanta, and St. Francis Hospital, Wichita, Kansas studied the specific nature of the antibody response to rabies vaccine. The scientists found that the present method of administering vaccine in daily injections produces too much antibody IgM. This antibody is not as effective as antibody IGG in the prophylaxis of rabies. In fact, IgM actually suppresses the production of antibody IgG. By modifying the timing of injections, the investigators suggest that IgG antibodies will be produced earlier in the course of treatment, thus increasing the efficacy of therapy and reducing the number of treatment failures.

## SUBHUMAN PRIMATE DIPLOID CELL LINES

Monkey diploid cell lines as candidates for use in human viral vaccine production were established this past year as the result of research carried out under contract to DBS. Although clinical experience with viral vaccines produced for many years from primary cell cultures has been uniformly successful, this project, under the direction of Dr. John C. Petricciani, DBS, was initiated in order to provide an alternative to the use of primary animal or human diploid cell cultures.

Priority has been given to development of cell lines derived from rhesus and African green monkeys, since there has been extensive experience with virus vaccines produced from cell cultures of these species with no evidence of untoward reactions. Diploid cell lines derived from fetal lung tissue have been developed from each species. The results have been sufficiently encouraging to pursue detailed characterization of these cell lines and to make them available for independent evaluation.

Characterization studies of two cell lines show them to have a finite life of approximately 50 culture passages while maintaining normal cell characteristics, including diploid condition of the chromosomes. Both cell lines are susceptible to a variety of viruses including polio, rubella, measles, and mumps.

Extensive tests have been made for the presence of adventitious agents in the cells. These include inoculation of the

cells into various laboratory animals and cell cultures, and electron microscopy and fluorescent antibody studies. Microbial agents (bacteria, fungi, mycoplasma, or viruses) have not been recovered. Tests in hamsters for tumor production have been uniformly negative. Studies involving transplantation of cultured cells into newborn animals of the same species from which cells were derived are now in progress. This system allows a latitude of testing and evaluation that does not exist with cultures of human cells.

The cells remain stable during extended periods of storage. No changes have been recognized in either cell line after six to twelve months of storage in liquid nitrogen.

The two cell lines thus far appear to meet requirements for economically feasible substrates for vaccine production in providing 1) adequate cell population, 2) rapid growth rate, and 3) yields of virus adequate for vaccine production.

## CHOLERA

Cholera is of increasing concern throughout the world. In the past two years, it has spread from Asia to the Middle East, to much of Africa, and even to parts of Europe. Vacationing French, Britons, and Scandinavians have brought the disease home with them and it is feared it may spread to the Western Hemisphere.

Although cholera can be controlled by improved sanitation, and treatment is now almost completely effective, these measures are often unobtainable in the contries at greatest risk and in rural areas.

For many years, the DBS has cooperated with the Pakistan-SEATO Cholera Research Laboratory in Dacca in conducting properly controlled field trials of available cholera vaccines. These vaccines, prepared from killed bacteria, have been shown to be useful, but protection is never complete and is of short duration--three to six months.

Control efforts have focused recently on the development of a cholera toxoid. This work is based on studies demonstrating that the diarrhea of cholera is caused by a toxin released by the Vibrio cholerae organism. A purified preparation of this toxin has been inactivated with formaldehyde, and studies relating to its safety and protective ability have been carried out in DBS and in other laboratories, including those of The Johns Hopkins, Maryland, and Texas universities. These studies have demonstrated that, although the toxoid was able to protect against cholera in human volunteers as well as in animals, severe local reactions occurred at the injection sites. These were found to be caused by reversion of the toxoid to active toxin after injection. For this reason, inactivation of toxin by other means is under study. It now appears that either heat or other chemical agents can produce a non-reverting toxoid which is still effective.

This year, Drs. John C. Feeley, Robert S. Northrup, and Francis V. Chisari of DBS have been assaying the stability, safety, purity, and antigenicity of these toxoids, and it is expected that small-scale trails in man will be possible in 1972. If these are favorable, larger field trials in areas where cholera is endemic can be started.

The SEATO Cholera Research Laboratory at Dacca is under the scientific direction of the NIH Cholera Advisory Committee, which also has functioned since 1959 to advise the Director of NIH on operations of the SEATO program. The committee chairman is the Scientific Director of the National Institute of Allergy and Infectious Diseases, which also conducts and supports cholera research activities. Other members of the Advisory Committee represent DBS, the National Communicable Disease Center, the National Institute of Arthritis and Metabolic Diseases, Army and Navy Medical Corps. the Agency for International Development, and several schools of medicine in the United States.

## MYCOPLASMA STUDIES

Mycoplasmas were first identified more than 70 years ago as the cause of pleuropneumonia of cattle, 10 years ago as the cause of pleuropneumonia or primary atypical pneumonia of man. Mycoplasmas have not been extensively studied. Recently, however, these micro-organisms have been implicated in other human diseases and presently an intense effort is being made to establish their role in human diseases of unknown cause.

It has been known for many years that mycoplasmas contaminate cell cultures. Since certain human viral vaccines are produced in cell cultures, these vaccines are subject to mycoplasma contamination. For this reason, the DBS requires a test for the presence of mycoplasma in all viral vaccines produced in cell cultures.

The DBS mycoplasma program, under Dr. Michael F. Barile's direction, is studying culture procedures for isolation of mycoplasmas in biologic materials.

Among the principal contaminants of cell cultures and vaccines are the bovine strains of mycoplasma. Since bovine sera are universally used for the growth of cell cultures, they have long been considered to be the source of mycoplasma contamination. Nevertheless, all previous attempts to isolate mycoplasmas from bovine sera, using standard procedures, had failed.

Recently, however, Dr. Barile and co-workers, using a new procedure, have succeeded in isolating mycoplasmas from contaminated bovine sera. A total of 121 mycoplasmas have been isolated from 424 lots of sera and five different species have been identified.

These studies have produced the first evidence that bovine sera is a major source of mycoplasma contamination of all cultures and

certain vaccines. The new culture procedure provides a more sensitive method for testing vaccine for the presence of mycoplasma contamination.

Grantees of NIAID and the National Institute of Child Health and Human Development (NICHD) have also reported a statistical correlation between maternal genital infection with T-strain mycoplasmas and low birth weight infants. Dr. Edward Kass and his associates, aware of reports of T-strain mycoplasma being isolated from fetal membranes after spontaneous abortions, carried out their prospective study of nearly 500 women at Boston City Hospital.

## TYPHOID VACCINE

Although the spread of typhoid fever can be controlled by sanitation, vaccination has been used since the early years of the century. The common vaccines are made of typhoid bacilli killed by treatment with acetone or heat and phenol. Field trials have demonstrated that the protective value is about 70 to 80 percent and lasts for 3 to 4 years following two immunizations. The acetone-inactivated vaccine is superior to the heat-phenol killed vaccine. Unfortunately, however, adverse reactions to both vaccines, due to endotoxin and other toxic substances in the bacterial cells, are encountered.

Control of typhoid vaccines has been a problem for biomedical regulatory agencies throughout the world because of the absence of suitable laboratory assay systems. The DBS has participated in international collaborative laboratory studies directed towards: (1) laboratory tests which will accurately reflect vaccine potency and reactivity for man, (2) improvement of vaccines by separation of toxic components from protective factors, and (3) elucidation of immune mechanisms of the disease.

A mouse protection assay system, established some years ago by Dr. Margaret Pittman, DBS, best reflects the relative protective potential of both the acetone-inactivated vaccine and the heat-phenol killed vaccine in man. Dr. K. H. Wong, DBS, in association with Dr. John C. Feeley and Dr. Pittman, has demonstrated that the protective activity of these two typhoid vaccines in mice is not only due to the long-recognized Vi antigen but to other factor(s) in the bacillus as well.

This year, DBS investigators, using a mild physical-chemical method, have isolated a Vi antigen which is about 200 times more protective for mice than the reference Vi preparation purified by an older method. Further work may resolve the long-existing controversy as to whether Vi antigen is necessary for protection in man.

## YELLOW FEVER VACCINE

Yellow fever vaccine has been licensed for many years and its effectiveness in inducing solid immunity to "wild" yellow fever virus is undisputed. However, the vaccine, which is produced in chicken embryos, was developed in the early 1940's when little was known about avian viruses in hens' eggs used to propagate the vaccine virus. Today, it is known that many chick embryos may be infected with avian leukosis virus.

Although there has been no evidence of any relationship between the avian viruses and human disease, DBS scientists have been working for years to purify the yellow fever "seed" viruses from which the vaccine is prepared. Dr. Nicola M. Tauraso, in collaboration with Drs. Edward B. Seligmann and Ruth L. Kirschstein, has developed primary and secondary yellow fever vaccine seeds free from avian leukosis viruses. The safety of these purified seed viruses has been established by tests in monkeys. Studies in man have shown the purified seed viruses to be safe and potent immunizing substances. The new seed strains will be routinely employed for vaccine production in the near future.

## PYROGEN TESTS

One of the required purity tests for many injectable (parenteral) biological products is the pyrogen test. If, during preparation, such a product becomes contaminated with bacteria, a substance known as endotoxin will develop which elicits a fever response (pyrogen reaction) when injected into man. The animal used in the standard pyrogen test for detection of endotoxin is the rabbit.

Like other animal assays, the rabbit fever test is expensive and time-consuming, and the response may vary. Moreover, environmental conditions, such as room temperature and extraneous noises, must be carefully controlled, since these conditions may influence rabbit temperatures.

In 1968, Levin and associates described a test tube assay for detecting endotoxin, based on the reaction between endotoxin and a lysate prepared from the circulating blood cells of the horseshoe crab. These studies indicate that this method of assaying endotoxin is faster, simpler, and potentially more sensitive than the currently used rabbit test.

This year, Drs. Edward B. Seligmann, Jr., and Donald Hochstein, DBS, have studied applicabilty of the horseshoe crab test to biological products. For the past several months, the test has been conducted in parallel with the rabbit pyrogen test. The correlation appears good. Further study is needed to determine 1) the method of preparation and storage of the lysate; and 2) the endpoint for a positive test.

Once these problems are resolved, the horseshoe crab test, with its speed, simplicity and sensitivity, could be successfully used for endotoxin detection in screening biological products.

## CANCER

The year 1971 was a significant one for cancer research. Far-reaching steps were taken by the President, Congress, and the National Cancer Institute (NCI) toward greatly expanding the national cancer research capability, and at the same time advances were being made in the laboratories and clinics of several institutes toward solving the complex problems of cancer.

## VIRUS-CANCER RESEARCH

The foundations for much of the progress made during 1971 in research on cancer viruses were the separate discoveries announced in June, 1970 of the enzyme, RNA-dependent DNA polymerase, also called "reverse transcriptase," by Drs. Howard Temin (McArdle Laboratories, University of Wisconsin) and David Baltimore (Massachusetts Institute of Technology). Until discovery of this enzyme, RNA, or ribonucleic acid, was considered to act in cells only as a servant chemical that usually was formed from a blueprint of DNA, or deoxyribonucleic acid, to carry out DNA's instruction in the cell. The new finding demonstrated that this enzyme enables RNA to be the blueprint for formation of DNA and thus control the cell's heredity. Discovery of reverse transcriptase enabled scientists to understand how certain animal viruses, whose cores contain RNA instead of DNA, can alter a cell's genetic material and consequently cause certain types of cancer in animals.

### Candidate Human Viruses

Because type-C RNA viruses have been strongly implicated in certain human cancers, scientists have worked to isolate such viruses from human tissues. Thanks to the discovery of reverse transcriptase, new techniques are now available to detect viral activity in human cancer tissue. These advances in study of cancer viruses have contributed to the biochemical evaluation of three candidate human cancer viruses discovered this year.

In the July 14, 1971 issue of Nature New Biology Drs. Elizabeth S. Priori, Leon Dmochowski, Brooks Myers and J. R. Wilbur of M. D. Anderson Hospital and Tumor Institute, Houston, Texas, under contract to the NCI Special Virus Cancer Program (SVCP), reported isolation of a type-C virus, named ESP-1, in cultured cells from a five-year-old American child with Burkitt's lymphoma, a relatively rare lymphatic cancer which usually affects the jaw and facial area of young African children.

First immunological studies of this virus by the M. D. Anderson group indicated that it did not cross-react with any known animal RNA type-C cancer virus and thus might be a human virus. Subsequent studies such as those reported in the September 10 issue of Nature by NCI scientists Drs. Wade P. Parks, Robert J. Huebner, and George J. Todaro, and others receiving support from the SVCP, indicated that the virus showed similarity to the leukemia virus of the mouse. Conversely, in November 1971, at the Symposium on Mammary Neoplasia

in Cherry Hill, New Jersey, Dr. Albert J. Dalton of NCI described what he believes to be structural differences between ESP-1 and every known subtype of mouse leukemia virus as viewed under the electron microscope.

Studies are now underway to determine whether the observed cross-reactivity between the mouse virus and ESP-1 virus may be due to an immunologic phenomenon common to the two species. Such a finding would indicate that the ESP-1 virus is at least unique and could be of human origin.

Early in December 1971, scientific teams working independently in the Washington, D. C. area and in Los Angeles, both cooperating in the NCI Special Virus Cancer Program, also reported what they believe to be new candidate human cancer viruses.

The discoveries were announced at the Children's Hospital of Los Angeles by Drs. Robert M. McAllister and Murray B. Gardner of the University of Southern California's School of Medicine and Dr. Robert J. Huebner, head of the Viral Carcinogenesis Branch, NCI, and in Washington, D. C., by Drs. Sarah Stewart and William Feller of Georgetown University. Both groups found the viruses, which may be the same, and are presumed to be cancer-causing in cells from cancers of skeletal muscle called rhabdomyosarcoma. The virus found by the USC group has been studied by several scientific teams within and supported by the NCI and found to be immunologically unique. It does not cross-react with any of the known animal cancer viruses. Scientists at Georgetown University now are evaluating their virus with similar tests. Further efforts are in progress, or planned, to determine whether these viruses are indeed of human origin, whether they occur primarily in human cells, and whether they are capable of causing cancer in animals.

## Viruses and Breast Cancer

Collaborating scientific teams supported by the NCI uncovered evidence strongly suggesting a viral cause of human breast cancer during 1971. In 39 percent of human milk samples from a group of Parsi women in Bombay, India, and 60 percent of American women at high risk to breast cancer, virus particles were found that were identical in appearance to viruses that cause breast cancer in the mouse. The study was reported in the February 26 issue of Nature by Dr. Dan Moore of the Institute for Medical Research, Camden, New Jersey, and collaborators in Camden, Detroit, and Bombay. These investigators also demonstrated that antibody from women with breast cancer neutralized the cancer-causing activity of the mouse virus. Antibodies are proteins formed to protect the body against foreign agents.

In the May 14 issue of Nature, Drs. Jeffrey Schlom and Sol Spiegelman of the Institute of Cancer Research, Columbia University, and Dr. Moore reported reverse transcriptase activity in human milk samples which contained virus particles. In November 1971, at the Symposium on Mammary Neoplasia, Dr. Spiegelman reported that his

group has been able to use a hybrid molecule, consisting of DNA from the mouse breast cancer virus and human RNA, as a probe for virus-specific information in human milk samples.

Also in November 1971 these scientists announced in the journal Science the development of a special test for simultaneous detection of reverse transcriptase and the high molecular weight RNA unique to RNA cancer-causing viruses. Biochemical detection of such cancer virus "footprints" in human tissue previously has been hampered by the low concentration of virus activity as well as by the presence of cell debris in the human material. Drs. Schlom and Spiegelman consider their test sufficiently simple, sensitive, and discriminating to signal the presence of a virus or viral components in biological fluids such as human milk. They devised their test with the mouse breast cancer virus and also have tested cat and chicken cancer viruses with the method.

## Biochemistry of RNA Cancer Viruses

A number of investigators have shown the presence of reverse transcriptase enzymes in all type-C RNA cancer viruses. In the May 1971 issue of the Proceedings of the National Academy of Sciences, Drs. Stuart Aaronson, Wade Parks, Edward Scolnick and George Todaro of NCI showed that in the various mammalian viruses these enzymes are immunologically related. These investigators and others are now exploring ways of inhibiting the activity of the enzyme in the virus by immunologic and chemical means as a potential approach toward controlling cancer.

In September at the Vth International Symposium on Comparative Leukemia Research in Padua, Italy, Dr. Raymond Gilden announced that he, Dr. Stephen Oroszlan, and Mr. David Bova of Flow Laboratories, Inc., Rockville, Maryland had developed antibody to the leukemia viruses of four different species of animals. The antibody developed by these scientists is now used extensively to analyze new mammalian cancer viruses that are isolated, including candidate human cancer viruses.

## "Slow Viruses"

Virus cancer research at NIAID also focused on two so-called "slow viruses" which infect sheep over long periods, causing slowly evolving, eventually fatal disease. Drs. Kenneth K. Takemoto and Lawrence B. Stone followed up their earlier observations that these viruses possess the special enzyme (RNA-dependent DNA polymerase or "reverse transcriptase") now believed common to all tumor-producing RNA viruses. The scientists injected cells transformed by the "slow-viruses" into newborn or irradiated mice and in less than two months, small tumors appeared at the injection sites. The study demonstrated, for the first time, that "non-oncogenic" RNA viruses which possess this special enzyme also have the ability to cause cancerous changes. This finding has important implications for both viral carcinogenesis and slow virus infections.

The tell-tale RNA-dependent enzyme has already become the target of anticancer drug research. Scientists have been testing compounds structurally similar to the antibiotics known as the rifamycins which inhibit the enzyme's activity. A NIAID research career development awardee, Dr. W. A. Carter, his co-workers at Johns Hopkins University, Baltimore, and representatives of the Upjohn Co. reported last year that the streptovaricins are particularly effective inhibitors of the RNA-dependent DNA polymerase of a mouse leukemia virus. The streptovaricins, unlike the rifamycins, are available in rather large quantities and their toxicity has already been assessed in preliminary studies. Dr. Carter and his group are now evaluating the effects of the streptovaricins on different polymerases in normal and leukemic white cells and are trying to determine whether cells transformed by tumor viruses can be changed back to normal under pressure of a polymerase blockade.

## DNA Viruses in Relation to Man

DNA viruses also have attracted much attention during the past year. In November, 1971, a team of NCI-supported scientists at the Johns Hopkins University, Baltimore, Maryland, led by Dr. Laure Aurelian, isolated herpesvirus type-2 (HSV2) from cervical cancer cells of a patient by growing them in highly alkaline broth in laboratory test tubes. Dr. Aurelian's work is the latest in a long chain of studies indicating that the relationship between HSV2 and cervical cancer is more than coincidental.

## Herpes-virus and Cancer

To test the assumption that there is an association between genital herpes and cervical cancer, scientists in the Section on Infectious Diseases, Perinatal Research Branch, National Institute of Neurological Diseases and Stroke (NINDS), established the Cebus monkey as an effective model for study of the problem.

Scientists will now be able to investigate a number of questions concerning genital herpes virus infection in humans. These include: the pattern of venereal transmission of genital HVH, and the role of the virus in producing spontaneous abortion, congenital malformation, and neonatal morbidity and mortality. The role of the virus in causing carcinoma of the cervix and chronic neurological diseases might be determined by long-term observation, and, finally, the efficacy of certain experimental drugs for the treatment of neonatal herpesvirus infection might be more rapidly determined in the Cebus monkey.

This study was reported by Drs. William T. London, David A. Fuccillo, and John L. Sever, Dr. Andre J. Nahmias, a grantee of the NIAID, and Dr. Louis W. Catalano, Jr., formerly with NICHD.

## Viruses and Hodgkin's Disease

In the September 10 issue of Nature, scientists at the Sloan-Kettering Institute for Cancer Research, New York City, working with partial support from NCI, reported association of viruses with

another type of human cancer, Hodgkin's disease. Drs. Magdalena Eisinger, F. Kingsley Sanders and associates were able to grow cells from patients with Hodgkin's disease which contained evidence of both RNA viruses and DNA viruses, the latter tentatively identified as herpes-type. These findings, while they are not evidence that these viruses cause Hodgkin's disease, are significant because they suggest the interaction of two types of viruses in the cause and course of the disease.

## Tumor-causing DNA viruses

In a project to learn about the lipid composition of cells, NINDS scientists, in collaboration with the NCI, have detected a definite chemical change on the surface of DNA virus-induced cancer cells.

Using tumor-causing DNA viruses to transform normal cells into cancer cells, the scientists found that there is a block in the synthesis of complex lipids on the surface of the cancer cells. They further identified the site of this enzymatic block in the synthesis of the complex lipids called gangliosides.

The investigators report that when tumor-causing DNA virus is invorporated into the genetic apparatus of a mouse cell, the ganglioside pattern on the cell membrane is changed. The gangliosides become more simple and do not produce the usual patterns of a normal cell because of the enzyme block.

This is the first time a defined chemical change has been identified on the surface of simian (monkey) virus 40 and polyoma (tumor) virus-transformed cells. The researchers hope to convert the cancer cell back to a normal cell by overcoming the genetic block.

These findings were reported by Drs. Roscoe O. Brady and Edwin H. Kolodny and Federico A. Cumar in the NINDS Laboratory of Neurochemistry and Dr. Peter T. Mora and Vivian W. McFarland in the Macromolecular Biology Section, Laboratory of Cell Biology, NCI.

## Genes and Cancer

During 1971 many Special Virus Cancer Program scientists reported results of experiments to understand the role of genetics in cancer. For example, at the NCI-supported Vth International Symposium on Comparative Leukemia Research in Padua, Italy in September, Dr. Hans Meier reported that he and Drs. B. Taylor and D. Myers of the Jackson Laboratory, Maine, had determined which gene controls gs-antigen expression in animal RNA cancer viruses. Antibody, protein that reacts specifically with this type of antigen, has proven invaluable in immunologic studies to identify animal cancer viruses.

## Effect of Chemicals on Cancer

In cancer virology, laboratory tools and models are necessities; hypotheses in this area can rarely be tested in man. Last year, NIAID scientists developed an important technique for leukemia research and reported results lending powerful support to the "oncogene" theory of cancer causation espoused by a number of NIH scientists -- that instructions for a leukemia virus may be present in unexpressed form in the DNA, or blueprint material, of seemingly normal cells. Using either of two chemicals -- 5-iododeoxyuridine (IUDR) or 5-bromodeoxyuridine (BUDR) -- Dr. Wallace P. Rowe and his associates activated mouse leukemia virus in mouse cell lines where none was formerly present. In fact, by means of these chemicals, it was shown that every cell in the cultures had the capacity to produce virus.

Extending this work, Drs. Stuart Aaronson, George Todaro, and Edward Scolnick of NCI showed by a similar experiment that each genetically identical cell of a mouse embryo cell line could be stimulated to demonstrate the presence of virus.

Several other NCI-supported scientists have also demonstrated ability to trigger chemically the formation of a type-C RNA cancer virus in animal cells. In March 1971, at the 24th Annual Symposium on Fundamental Cancer Research, Houston, Texas, Drs. Robert J. Huebner and Gary J. Kelloff, NCI, and several groups of NCI contractors reported results of experiments to develop ways to make quick and precise identification of carcinogens, or cancer-causing chemicals, in the environment. One such study, by Dr. Aaron E. Freeman of Microbiological Associates in Bethesda, Maryland, showed in test tubes studies that smog residues can trigger transformation, or cancerous changes, in animal cells chronically infected with various animal leukemia viruses. When the same concentration of smog residue or virus was used alone, transformation did not occur, suggesting the need for both the chemical and the virus interaction to produce the cancerous changes. Other scientists in the study found similar transformation of laboratory-grown cells for five other chemical carcinogens. This work, coordinated by the Viral Carcinogenesis Branch of the NCI, could lead to the development of highly sensitive test tube assays of suspected carcinogens.

## A New Model For Carcinogenesis and Teratogenesis

An experimental animal capable of developing in the laboratory the special forms of cancer common to childhood would be of considerable value as a tool in research into this leading cause of death in children. The American marsupial, the opossum, is of interest in this regard because its young are born in a fetal state (12 3/4 days gestation). This permits observation of the development of the young during the time when a mammalian fetus is *in utero*.

A continuing study of the response of the opossum exposed by the mouth at several stages of development from birth to three months to a chemical carcinogen (any cancer-producing substance) indicates

that this animal may have a unique susceptibility to tumor formation. These experiments have already shown that tumors rarely produced in animals in the laboratory have been produced in the opossum. They originated early in the development of liver, kidney, lung, eye, and jaw of the opossum. Several forms of skin cancer have also developed in these animals. In addition birth defects in the form of kidney cysts are quite common in the animals exposed to the carcinogen, suggesting that the opossum may also be useful in studying the relationship between malformations and cancer.

The apparent lack of immunologic competence at birth makes the animal suitable for experimentation regarding the induction of tumors with human cancer viruses and for the transplantation of human tumors.

## THIRD NATIONAL CANCER SURVEY

In September 1971 the NCI published a preliminary report on the Third National Cancer Survey, analyzing cancer statistics in eight large metropolitan areas and one entire state. This report gives the cancer incidence for 1969 and indicates the following trends: (1) The overall incidence of cancer in men is increasing, a trend particularly marked among blacks, while in women it is decreasing. (2) The incidence of lung cancer has doubled since 1947 in both men and women of both races. (3) The incidence of cancer in blacks is substantially higher than in whites, a difference particularly large between black and white men. It is not likely that these differences in cancer incidence arise from genetic causes, but they are more likely to be due to socio-economic factors.

Data for the survey were collected in Iowa, Detroit, Minneapolis-St. Paul, Pittsburgh, Atlanta, Birmingham, Dallas-Fort Worth, Denver, and San Francisco-Oakland, with a combined population of over 20 million residents. A total of 61,409 new cancers were diagnosed in this population during 1969. Statistics generalized for the entire U. S. population indicate that, in addition to minor skin malignancies, 610,000 new cases of cancer were diagnosed that year.

The Third National Cancer Survey, a sequel to the Ten Cities Surveys of 1937 and 1947, is being conducted by the NCI Biometry Branch in conjunction with medical schools and associations, health departments, voluntary health agencies, and other organizations. In addition to data on cancer incidence, these groups are collecting information on the social and economic effects of cancer and the medical care needed by cancer patients. Preliminary attempts at evaluating the data have only begun. The final report, which will include data for the years 1969 through 1971, is due in 1973.

## CHEMICALS AND CANCER

With increasing chemical pollution of the environment, concern about carcinogens, or cancer-causing chemicals, has been growing. This concern was reflected in some of the important NCI activities and research during 1971.

### Evaluating Carcinogenic Hazards

Staffs of the Food and Drug Administration and NCI Carcinogenesis area held a joint conference on the Criteria for Evaluation of Carcinogenic Hazards of Drugs in December 1971. The conference was coordinated by Dr. W. B. D'Aguanno of the FDA and Dr. Umberto Saffiotti, NCI's Associate Scientific Director for Carcinogenesis. Participants included representatives of the Pharmaceutical Manufacturers Association, the President's Science Advisory Committee, the National Academy of Sciences and several other scientific organizations. Plans were proposed for increased cooperation between the FDA and the NCI in monitoring hazardous drugs and food constituents.

The first annula meeting of the Lung Cancer Segment of the Lung Cancer Program in Carcinogenesis was held November 3-5, 1971. The Program supports studies in the causes and biological mechanisms of lung cancer as a basis for preventing this disease, which kills 68,800 Americans each year. The participants, NCI scientists and those under contract to the NCI, spoke on multiple factors in respiratory carcinogenesis and cellular and biochemical studies.

As part of a continuing effort to evaluate chemicals in the environment, the NCI awarded a contract in June 1971 to the Stanford Research Institute (SRI) to study in animals the effect of environmental chemicals in combination with other chemicals. As a result of another study, initiated in November 1970 by the NCI at the SRI, investigators announced in December 1971 the feasibility of a new information system called CHRIS (Cancer Hazard Ranking and Information System) to rank chemicals according to their relative cancer-causing potential for man. To date, the SRI scientists have used the system to assess a group of air and water pollutants, soaps and detergents, food chemicals, and agricultural chemicals. It can continually re-evaluate the ranking of hazardous chemicals as more data are added.

A fourth volume in the series "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" became available in early December 1971. The new publication, prepared under an NCI contract by John I. Thompson and Company, Rockville, Maryland, includes reports for the years 1968 and 1969. The 650-page volume continues work formerly undertaken by Drs. Jonathan L. Hartwell and Phillip Shubik in their first three surveys of the scientific literature through 1960. NCI plans at least two additional supplements, 1961-1964 and 1965-1967.

## N-nitrosamines in Cigarette Smoke Condensate

NCI-supported investigators, Drs. Donald Johnson and John W. Rhoades of the Southwest Research Institute, San Antonio, Texas, announced in December 1971 that they had identified a type of cancer-causing chemical called N-nitrosamines in cigarette smoke condensate. These chemicals have long been considered as possible components of tobacco smoke condensate, but the investigators are the first to demonstrate their presence. The scientists also reported that the amount of N-nitrosamine in cigarettes depends on the variety of tobacco used and the amount of nitrogen in the soil in which the tobaccos were grown. This study is part of an effort to complete the profile of chemical constituents of cigarette smoke condensate, so that cancer-inducing substances can be identified and eliminated.

## Occupational Hazards

A study reported in the April 16, 1971 issue of Nature by Drs. E. K. Weisburger, J. M. Rice, and J. H. Weisburger indicates the carcinogenicity in rats of two industrial chemicals, propylene imine and propane sultone. Propylene imine, an important chemical intermediate with many applications in the production of polymers, coatings, adhesives, textiles and paper finishes, caused tumors in a wide range of organs in the test animals. Propane sultone, a chemical with many potential uses in the detergent, textile, rubber, pharmaceutical and agricultural chemical industries, was equally carcinogenic. The investigators emphasized the need for adequate precautions in handling these compounds because of their potential deleterious effects in living systems.

Another widely used industrial chemical, bis (chloromethyl) ether, was also identified as a potential occupational hazard. In a preliminary report in the Archives of Environmental Health in August 1971, Prof. Sidney Laskin, an NCI contractor at the New York University, and his co-workers described the development of tumors of the lung and nasal cavity in rats that inhaled bis(chloromethyl) ether at extremely low concentrations.

# CANCER DETECTION

In 1971 steps were taken to further the development of tests for the detection of some of the major forms of cancer in early, presymptomatic stages. In July, funding of an NCI contract to evaluate an early detection test for cancers of the colon and rectum was announced. Contract investigators at Tufts University School of Medicine in Boston, Dr. Robert Schwartz and his colleagues, are examining the specificity of a test, developed by Dr. Phil Gold of Canada, which measures carcinoembryonic antigen (CEA), a substance which has been found in the blood of patients with these cancers and may be a sign of very early cancer.

A project to improve survival of lung cancer patients was initiated at the Mayo Clinic, Rochester, Minn., under an NCI contract. Dr. Robert S. Fontana and his co-workers are screening men at high risk of developing lung cancer due to smoking. They are using chest

X rays and microscopic examination of cells obtained from deep cough sputum, the only procedures so far proved useful in detecting presymptomatic lung cancer. Although the techniques used are not new, this will be the first large-scale surveillance of persons at high risk to lung cancer studied with these techniques. The investigation will also evaluate other early detection methods and procedures for locating tumors too small to be seen on an X-ray.

Preliminary findings from an NCI contract-supported study on the value of a breast cancer detection method were published in the March 15, 1971 issue of the Journal of the American Medical Association. The investigators, Mr. Sam Shapiro, Dr. Phillip Strax, and Dr. Louis Venet, based their report on data from 62,000 women between the ages of 40 and 64 enrolled in the Hospital Insurance Plan of Greater New York. Half the group was offered four annual breast examinations with mammography (a special X-ray technique) and regular clinical procedures. The other half followed their usual practices in receiving medical care and did not participate in the planned annual screening program. Results indicate 40 percent fewer deaths due to breast cancer among women screened with mammography and clinical examination, compared with breast cancer deaths occurring among the unscreened group. Based on this preliminary report on the effectiveness of such a screening program, the investigators recommended modifications in mammography and training of more medical personnel in order to screen large portions of the population for breast cancer.

## CANCER DRUGS

During 1971 research on cancer chemotherapy, the drug treatment of cancer, continued to be an important part of NCI's effort to control cancer. Research efforts ranged from the search for potential new drugs to finding the most effective means of using the drugs now available.

### Development of New Cancer Drugs

A total of 14,226 new compounds was tested by NCI in 1971 as potential anti-cancer agents. These included approximately 9,000 synthetic chemicals and 5,000 natural products from fermentation, plant, and animal sources.

The largest numbers of tests were performed using mice with leukemia L-1210, a tumor model found to be the most reliable system for predicting the response of human cancer to drug therapy. The number of tests with these mice increased 17 percent in 1971 compared to 1970.

The next most frequently used tumor model was the P-388 mouse leukemia, similar to the L-1210 but able to detect the activity of smaller amounts of crude material. It was therefore used to test some of the natural products. The total number of tests in 1971 with the various tumor models and cell systems was 13 percent higher than in 1970.

As a result of such initial screening against rodent cancers, nine materials showed sufficient anti-tumor activity to be selected by NCI scientists for pharmacology-toxicology studies in large animals. Such studies are prerequisite to drug evaluations in human cancer, in order to assure that the test compounds will be relatively non-toxic and safe to use. Moreover, they help to determine the starting dose for human drug administration, which usually begins in NCI-sponsored drug evaluations at one-tenth the dose tolerated by dog or monkey (whichever is less).

During 1971 seven compounds passed the NCI's stringent tests in animals for pharmacologic evidence of activity and safety, and were accepted for study against human cancer. The materials selected for these important tests are methyl-CCNU; cis-diamminedichloro-platinum; ICRF-159; and 9-B-D-arabinofuranosyl- 9H-purine-6-thiol; chromomycin A3; estriol; and gallium nitrate. During 1971 the National Cancer Institute filed Investigational New Drug applications with the Food and Drug Administration to conduct clinical studies of the first four of these drugs plus 1-propanol,3,3'-iminodi-,dimethanesulfonate hydrochloride; 5-azacytidine; iphosphamide; and rifamycin SV. Then the painstaking, long-term clinical studies were begun to determine whether or not the materials will offer cancer patients therapeutic therapeutic benefits not now available through conventional methods of treatment.

## Clinical Evaluation of New Cancer Drugs

Promising results on two relatively new anti-cancer drugs were reported this year. Adriamycin, an antibiotic developed by the Farmitalia Research Laboratory, an Italian firm, has been found effective against a wide range of human cancer, including some of the solid tumors seldom responsive to drugs. Two groups of NCI grantees, participants in the Acute Leukemia Cooperative Group B and the Southwest Cancer Chemotherapy Study Group, reported results with this drug in the October 1971 issue of Cancer. They studied a total of 153 patients with advanced cancer, some of whom had received prior chemotherapy. Among the patients responding to the drug were those with acute lymphocytic leukemia (a cancer occurring primarily in childhood), acute myeloblastic leukemia (a leukemia usually found in adults), neuroblastoma (tumor of embryonic nerve cell tissue), Wilms' tumor (childhood kidney tumor), reticulum cell sarcoma (a cancer of the lymphatic system), Ewing's sarcoma and osteogenic sarcoma (two types of bone cancer), and cancers of the lung, bladder, and breast. The patients who responded experienced either a reduction in the size of their tumor or complete, though temporary, disappearance of their cancer. Further study will determine the most effective dosage and schedule for adriamycin and the cancers most responsive to this drug.

The second promising drug, trimethylcolchicinic acid (TMCA), a compound that inhibits cell division, was one of a group of drugs tested in a study of 73 patients with advanced malignant melanoma, a usually fatal cancer that my develop from a pre-existing mole. NCI grantees, Drs. F. Deborah Johnson and Edwin M. Jacobs of the Cancer Research Institute, San Francisco, reported their 10-year study (January, 1960--December, 1969) in the June, 1971 issue of Cancer.

TMCA produced significant responses comparable or superior to any other drug or combination of drugs used. In addition, TMCA was easy to administer and was tolerated reasonably well by a majority of patients.

## Treatment with Combinations of Drugs

With the use of combinations of anti-cancer drugs, long-term survival has been achieved in patients with advanced stages of Hodgkin's disease, a cancer of the lymph system. NCI scientists Drs. George Canellos, Robert C. Young, and Vincent T. DeVita discussed the survival of a group of patients treated at the Institute between 1964 and 1967 at the Hodgkin's Disease Symposium held in October 1971 in St. Louis, Missouri under the sponsorship of the Cancer Clinical Investigation Review Committee and the NCI Clinical Investigations Branch. (Advanced Hodgkin's disease is treated with drug therapy; early-stages of the disease are treated by radiation, as discussed below.)

Of the 43 patients in the study group, 35 achieved complete remission, that is, apparent disappearance of their disease. Of these 35 patients, 24 are now alive without evidence of cancer 4 to 7 years after the start of treatment with a combination of vincristine, procarbazine, prednisone, and an alkylating agent. Fifteen of these 24 patients have remained in complete remission with no need for further treatment after initial chemotherapy. Since these 24 patients represent 69 percent of the original 35 patients who achieved complete remission of their disease, complete response to initial combination therapy may forecast long-term survival.

Combination chemotherapy initiated in 1967 at the NCI for patients with advanced lymphosarcoma, another type of cancer of the lymph system, is achieving higher survival rates than treatment programs previously reported. Dr. Charles M. Bagley and his co-workers discussed the use of cyclophosphamide, vincristine, and prednisone in combination at the annual meeting of the American Association for Cancer Research in Chicago in April 1971. Of the 35 patients in the study, 20 (57 percent) had complete remissions of lymphosarcoma, 12 (34 percent) had partial remissions, and 3 (9 percent) failed to show any response. Of the 20 patients who achieved complete remissions, 18 had remained in a disease-free state for 1 year at the time of reporting. In addition, 28 patients (79 percent) were alive 1 year after initial treatment and 26 patients (73 percent) remained alive 2 years after treatment.

## Treatment of African Lymphoma

Cyclophosphamide is a drug known to be effective against Burkitt's lymphoma, a cancer of the lymph system which has been studied primarily among African children. Study of the drug over the past four years at the Lymphoma Center in Uganda, where the disease appears to be endemic, has revealed that the outlook for survival and recovery from this disease is independent of initial treatment and depends on the extent of the cancer when medical treatment begins. Results of this continuing study were reported

by Dr. John Ziegler, NCI scientist and director of the Uganda Cancer Institute, at the NCI-sponsored Vth International Symposium on Comparative Leukemia Research in Padua, Italy, in September.

Since 1967, 130 patients with Burkitt's lymphoma have been treated with cyclophosphamide at the Center. Over 90 percent of this group achieved complete disappearance, temporarily at least, of their cancer. Of those with localized disease (usually facial tumors) 76 percent survived at least two years and of those with abdominal tumors 63 percent survived at least two years. Patients with involvement of the central nervous system on admission had a survival rate of 52 percent at one year. The poor survival rate of this group reflects in part the present inability to cure malignant invasion of the brain.

### New Leads in Cancer Chemotherapy

In the February 1971 issue of the Proceedings of the National Academy of Sciences, Drs. George Johnson, Ira Pastan, and co-workers reported that adenosine 3',5'-cyclic monophosphate (cyclic AMP) and its derivatives partially restore normal cell characteristics to certain animal and human cancer cells grown in tissue culture. Cyclic AMP is found in all cells and mediates the action of many hormones. It was discovered in 1956 by Dr. Earl W. Sutherland, an NIH-supported scientist who won the Nobel Prize for Medicine in 1971.

Drs. Johnson and Pastan reported in the December 1971 issue of the Journal of the National Cancer Institute that prostaglandins, hormone-like compounds which also act with the help of cyclic AMP, caused changes in the structure and growth of certain animal cancer cells similar to the changes produced in the earlier study with cyclic AMP derivatives. These findings suggest that at least some actions of prostaglandins on cancer cells are due to increased levels of cyclic AMP stimulated by the presence of the prostaglandins. Clarification of the relationship between cyclic AMP and prostaglandins may possibly lead to control of tumor growth in man by administration of either of these two compounds in order to raise the level of cyclic AMP in the cancer cells.

## TREATMENT WITH SURGERY AND RADIATION

### Cryosurgery

In recent years cryosurgery, the destruction of tissue by freezing, has been used in the palliation of readily accessible human cancers of the mouth, pharynx, prostate, and rectum. As a curative treatment it has been limited to skin cancers. But recent studies have encouraged investigation into cryosurgery as a potentially useful form of treatment for other cancers. In one such study, cryosurgery was found to eradicate liver cancers in rats, without adverse effects to the animal as a whole. These findings were reported in the November 1971 issue of Cancer by NCI scientists Drs. H. Bryan Neel, III, Alfred S. Ketcham, and William G. Hammond.

## Surgical Treatment of Cancer of the Cervix

A review of NCI's clinical experience during a 15-year period with cancer of the uterine cervix, one of the most common forms of cancer in American women, was completed in 1971. Various aspects of this experience were reported by Dr. Alfred S. Ketcham and his colleagues in the May 1971 issue of Obstetrics and Gynecology and the November 1971 issue of Cancer.

This experience has resulted in the establishment of guidelines for treatment of patients with unusually large or incurable untreated tumors and of patients who have had previous treatment failure. Now such patients often can be treated with radical surgery safely and sometimes can be cured.

From 1954 to 1969, 162 patients with far-advanced cancer were treated with pelvic exenteration, the surgical removal of the pelvic organs and lymph nodes, resulting in a cumulative five-year survival of 38 percent. During the same period, another 84 patients with less advanced stages of cancer, were treated with radical hysterectomy, the surgical removal of the uterus and all or part of the vagina plus the pelvic lymph nodes ( a more limited operation than pelvic exenteration), with a cumulative five-year survival of 83.5 percent.

## Classification of Hodgkin's Disease Stages

At a conference in April in Ann Arbor, Michigan, on Staging of Hodgkin's Disease, a new method of classifying the stages of the disease was proposed to assist physicians in choosing the most effective therapy. In the November 1971 issue of Cancer Research, this new classification, or staging, method was reported by Dr. Paul P. Carbone of NCI and other members of the Hodgkin's Disease Classification Committee appointed by the American Cancer Society.

The new staging method is based on two systems of classification --clinical and pathological--rather than on the single clinical system of the classification method used since 1965. The need for a pathological system became apparent during the past few years when laparotomy (surgical exploration of the abdomen) was proposed as part of the diagnostic evaluation of Hodgkin's disease patients. Laparotomy allows a physician to obtain tissue samples for microscopic study (biopsy) from organs and sites not accessible to palpation and not clearly defined by X-ray examination.

The new staging method promises to improve the outlook for Hodgkin's disease patients, particularly for those with localized cancer in areas outside the lymph system. More effective comparisons of data from different cancer centers will also now be possible.

## IMMUNOLOGY AND CANCER

### Structure of a Human Antibody

In a further step toward understanding the way the body fights diseases such as cancer, Dr. D. R. Davies and his colleagues in the NIAMD and Dr. William Terry of the NCI presented new data on the three-dimensional structure of a human antibody. Antibodies are proteins formed as part of the immune response against foreign agents called antigens, composed, for example, of cancer-related proteins. According to present concepts, a person may develop cancer when his immune system is no longer effective in recognizing as foreign the antigens of cancer cells and therefore does not proceed to deactivate the harmful cells.

In two reports in the December 10 issue of The Journal of Biological Chemistry, the investigators and their colleagues described the use of X-ray diffraction and electron microscopy to study the structure of a crystal of a human antibody, a protein called gamma G1 immunoglobin. These preliminary studies offer direct evidence that the overall outline of the molecule has either a T or Y formation. Further experiments are planned to determine the three-dimensional structure in greater detail and thus provide more information on the way antibodies interact with antigens.

### Specific Antigens on Leukemia Cells

During the year two groups of NCI scientists presented evidence suggesting that leukemia cells contain specific antigens that make those cells distinguishable from normal cells. Establishing the presence of these antigens is an important step in developing therapy based on principles of immunology as well as detection methods. Such antigens may indicate that an individual has early, presymptomatic leukemia or that he has been exposed to the causative agent, possibly a virus, and may later develop the disease. These antigens thus may also serve as a marker for the causative agent.

One group of investigators, Drs. Eugene B. Rosenberg, Ronald B. Herberman, Paul H. Levine, and John R. Wunderlich reported in April, at the annual meeting of the American Association for Cancer Research (AACR), on a study of the reaction of cells of leukemia patients and their normal identical twins to cells of family members and unrelated normal individuals. Identical twins, who have compatible tissue types, were studied as controls to minimize the possibility that observed reactions were due to differences not attributable to leukemia. Presence of antigen was indicated in test tube studies of white blood cells called lymphocytes. In four of six families, lymphocytes from family members reacted against lymphocytes from the leukemic patient but not from the normal identical twin.

In December 1971, Dr. Dean Mann and his colleagues at the NCI reported in the journal Science further evidence for the existence of a specific antigen on human leukemia cells from a different type of test. Using antiserum from rabbits immunized against Burkitt's

lymphoma cells, they tested the peripheral white blood cells of 15 patients with either acute lymphocytic or acute myelocytic leukemia, 41 of their relatives, and 527 unrelated persons. White blood cells of 8 of 15 leukemia patients and 4 of their relatives showed a positive reaction to the antiserum, suggesting the presence of a common immunologic factor among the patients and some of their close relatives. Most of the patients whose cells did not react were in remission, a temporary disease-free state. No reaction was observed with random individuals.

Evidence for the existence of specific antigens on leukemia cells, as indicated in these studies, suggests that it may be possible to detect persons exposed to factors that cause leukemia in order to plan for preventive or therapeutic measures.

## Experimental Immunotherapy

Evidence that may lead to treatment of cancer patients with a type of immunotherapy called transferred immunity was presented in April at the AACR meeting. From their experiments with guinea pigs, whose immune reactions are similar to those in man, NIH scientists Drs. Irwin D. Bernstein, Daniel E. Thor, and Herbert J. Rapp concluded that successful transfer of cancer immunity appears to be a two-step process. It had previously been thought that immunity could be transferred from immunocompetent animals by injecting some of their lymphocytes, capable of reacting to a specific antigen, into a recipient whose immune system was unable to provide these specific immune cells. The immunocompetent cells would then deactivate the harmful antigens such as those found on cancer cells. Deactivation of cancer-related antigens by lymphocytes is part of the immune response of the body and is now considered an important mechanism in preventing the development of cancer. In their animal studies, the investigators found that there must also be a supply of non-specific infection-fighting cells (macrophages) contributed by the recipient of the transferred immunity for rejection of cancer tissue. Since present findings indicate that macrophages are absent or impaired in patients with advanced cancer, the investigators suggest that stimulation of the production of these cells in cancer patients might increase the effectiveness of transferred cancer immunity.

## How Tumors Grow

NIH-supported scientists at the Harvard Medical School and Children's Medical Center, Boston, interested in the immunological aspects of cancer, have found that a tumor itself contains an apparently distinctive agent which stimulates development of the capillaries necessary for continuation of its own growth. This substance, which the investigators have named TAF, or tumor angiogenesis (development of blood vessels) factor, has been isolated from both human and animal solid tumors. Thus far, it has not been found in non-malignant tissues.

The Boston scientists (Drs. J. Folkman, E. Merler, C. Abernathy,

and G. Williams) suggest that, if TAF were to be blocked, tumor growth might well be arrested at a very early stage. Since, unlike normal tissue, only a growing tumor demands new capillaries for its continued growth, an anti-TAF agent (assuming one can be developed) would not be harmful to normal tissues but would have the potential to deprive cancer cells of a vital means for their continued expansion.

## HEART, BLOOD VESSEL AND LUNG DISEASES

Advances against many categories of disease affecting the heart, blood vessels and lungs were made in research supported and conducted by NIH.

### HYPERTENSION

Hypertensive subjects under treatment with blood-pressure-lowering drugs commonly experience a slight reduction in blood volume, according to Dr. Harriet P. Dustan and co-workers at the Cleveland Clinic, and this may be an important factor in their blood-pressure response to therapy. The research was supported by NHLI.

The scientists investigated blood volume as a potential factor in both "good responders" and "poor responders" to antihypertensive regimens.

"Poor responders" had blood pressures averaging 165/105 despite having followed a regimen of antihypertensive and diuretic therapy. Their plasma volume was slightly in excess of normal (about 109%). Intensified diuretic therapy in this group reduced their plasma volumes to 93% of normal and their average blood pressure to 145/93.

"Good responders" to antihypertensive therapy (blood pressures averaging 136/85 or less) consistently exhibited plasma volumes less than 85% of normal.

The results indicate that plasma volume is a critical factor affecting the results of drug regimens for blood-pressure control, and that reduction of an excessive plasma volume by prompt and vigorous antidiuretic therapy may establish control of blood pressure levels even in "poor responders."

#### Sensitivity to Pressor Agents

There is evidence that certain patients with hypertension are more sensitive than normal subjects to agents that constrict blood-vessels, such as angiotensin or norepinephrine. It is not known whether this heightened sensitivity is a critical factor in the development of their hypertension or in its maintenance, but it would certainly seem to predispose them to development of the disease and might well aggravate its course.

Drs. Hiroshi Mizukoshi and A. V. Michelakis, of the Vanderbilt University School of Medicine, report that blood plasma

from hypertensive subjects contains a factor--probably a hormone and possibly produced by the kidney--that does intensify the blood-vessel constriction and blood-pressure increase elicited in mice by angiotensin or norepinephrine.

Plasma from normal subjects did not amplify the blood-pressure response of rats to graded doses of these pressor substances. In contrast, pretreatment with plasma from subjects with essential hypertension increased the subsequent blood-pressure response to angiotensin by 30-40% and the response to norepinephrine by 28-30%. Pretreatment with plasma from subjects with renal hypertension increased responses to both agents by about 20%.

Subsequent studies, also supported by NHLI, indicated that the active principle was present in high concentrations in plasma collected from the renal veins of patients with renovascular hypertension, but was not present in renal plasma from normal subjects. This suggests that the sensitizing substance is produced by the kidney.

The sensitizing factor has not yet been identified, but the results thus far indicate that it differs from any known pressor agent.

## Combination Drug Therapy

Drs. Thomas Gottlieb and Charles Chidsey, of the University of Colorado Medical Center, report that the new blood-vessel dilator Minoxidil (Upjohn), when used in combination with certain other drugs, provides excellent blood-pressure control in hypertensive patients who do not respond adequately to other antihypertensive combinations.

All patients participating in this NHLI-supported study were initially on the diuretic agent hydrochlorothiazide and propranolol, a drug that helps lower blood pressure by reducing heart rate and reducing the workload on the heart. To each of these combinations was added either hydralazine or Minoxidil, both of which tend to lower blood pressure by dilating constricted blood vessels.

On propranolol and hydrochlorothiazide only, the patients' blood pressures averaged 193/128. Addition of hydralazine reduced this to 175/113, but the reductions achieved by adding Minoxidil to the regimen were substantially greater and average blood pressure fell to 139/93.

Further studies indicated that the antihypertensive action of Minoxidil developed rapidly and persisted for more than 24 hours--longer than that of most blood-vessel dilators--despite the fact that the drug is excreted rapidly by the kidneys.

The grantees conclude that the combination of propranolol, diuretic therapy, and Minoxidil has much to recommend it in the treatment of refractory hypertension.

## CARDIAC DISEASES

Drs. M. L. Marcus, L. E. Grauer, G. Krishna, and Stephen E. Epstein, of the NHLI Cardiology Branch, report that the powerful heart stimulant norepinephrine may increase the tempo and vigor of heart-muscle directly rather than through the chemical intermediate cyclic-AMP.

Recent evidence has indicated that cyclic-AMP may be a common denominator in the disparate actions of a large number of hormones on susceptible tissues: the release of adrenal cortical hormones by ACTH from the pituitary, the increased water reabsorption by the kidney in the presence of antidiuretic hormone, the release of free fatty acids from adipose tissue or of glucose from the liver in response to epinephrine or other hormones. All these are accompanied by increased concentrations of cyclic-AMP in the affected organs or tissues. A current hypothesis holds that various hormones act primarily to increase the production of cyclic-AMP from the high-energy substance ATP in susceptible tissues. In turn, the cyclic-AMP enforces the hormonal directives, then, its job done, the cyclic-AMP is rapidly inactivated by enzymes called phosphodiesterases.

It has been generally believed that the effects of catechol amines (norepinephrine and epinephrine) on the heart were also mediated by the amine-induced formation of cyclic-AMP, since heart-muscle concentrations of this substance are commonly increased by these amines. However, the NHLI studies, which plotted myocardial concentrations of cyclic-AMP against increases in heart rate or contractility in response to catechol amines, indicated that the cardiac stimulatory effects of these amines preceded any substantial increase in cyclic-AMP concentration and, in some instances, occurred while cyclic-AMP levels were falling.

The observed lack of correlation between cyclic-AMP levels and changes in heart rate or contractility raises questions concerning the role (if any) of cyclic-AMP as mediator of the myocardial stimulatory effects of catechol amines.

### Acute Heart Failure Drug

The drug phentolamine may be a clinically useful agent in treating acute heart failure resulting from heart attacks or other causes, according to Drs. W. B. Hood, Jr., J. B. Singh, W. H. Abelmann, and B. J. Polansky of the Boston City Hospital.

Phentolamine has two primary effects on the circulatory system: one is to relax constricted blood vessels by blocking alpha-receptors in the blood-vessel wall; the other is to increase heart rate and contractility by stimulating cardiac beta receptors. Both actions are likely to be salutary in acute heart failure, where both heart output and bloodflow to the organs and tissues of the body are often compromised.

The NHLI-supported scientists studied the effects of

phentolamine in dogs with heart failure developing after acute heart attacks (myocardial infarction) induced by blockade of one of the major coronary arteries. Phentolamine infusions produced moderate increases in heart rate and restored heart output to levels only slightly below pre-infarction values. Blood pressure in the aorta (which the heart must pump against in expelling blood) fell slightly, and there was improvement in certain other indices of heart performance.

The investigators conclude that phentolamine--whose effects resemble that of another promising cardiac stimulant, the pancreatic hormone glucagon--may have value in coping with heart failure developing in the wake of acute heart attacks. Possibly enhancing this value is the fact that the drug tends to counter certain abnormalities of heart rhythm that can pose a severe threat to the patient's life after an attack.

## Digitalis in Pregnancy

In pregnant women receiving digitalis for various heart conditions, this drug readily crosses the placenta to produce plasma levels of digitalis in the fetus that closely approximate those of the mother, according to Drs. M. C. Rogers, J. T. Willerson, Allan Goldblatt, and T. W. Smith, of Massachusetts General Hospital, Boston.

Eleven women receiving 0.25 mg./day of digoxin for rheumatic heart disease were studied at delivery. Their blood digoxin levels averaged 0.6 nanogram (billionths of a gram) per milliliter. The digoxin concentration in cord bloods and in blood samples drawn from their infants 5 hours after delivery averaged 0.5 ng./ml.

Repeat determinations were done in 5 of the mothers 1 month later, at which time their digoxin levels were substantially higher (1.1 ng/ml) than at term, even though their daily dosage of the drug had remained the same.

The investigators conclude that 1) when digitalis is administered during pregnancy, the fetus is digitalized to about the same extent as the mother; 2) the newborn and infants tolerate the drug well; 3) changes occurring during pregnancy may increase the dosage of digitalis required to maintain therapeutic blood levels of the drug in expectant mothers; and 4) intrauterine digitalization is feasible for the fetus whose well-being may be threatened by heart-rhythm disturbances or other drug-correctable conditions that may be detectable before birth.

## Lead and Heart-Muscle Degeneration

Toxic levels of lead, picked up by bootleg whiskey during distillation in primitive stills, may be an important factor in the heart-muscle degeneration sometimes found among alcoholics, according to Drs. S. K. Asokan and Martin J. Frank of the Medical College of Georgia.

In these studies, cardiac performance in 11 moonshine drinkers under treatment for alcoholic heart problems was studied before administration of calcium EDTA and at frequent intervals thereafter. Calcium EDTA is a chelating agent that can hasten the excretion of certain metals by combining with them to form salts more easily handled by the kidneys.

After chelation therapy, the heart output of these patients, initially low, rose by an average of 25% and similar dramatic improvement was observed in other indices of heart performance. Over the next 24 hours, the urinary output of lead was abnormally high in all patients, exceeding 600 micrograms.

Five control patients with heart-muscle dysfunction not of alcoholic origin received the same course of calcium EDTA therapy, but neither experienced the improvement in heart performance observed in the moonshine drinkers nor did their urinary lead output rise above normal.

## Deaths From Aerosol Gases

A number of deaths have been reported among adolescents attempting to "turn on" by inhaling aerosol propellant gases, chiefly Freon-12. Investigating possible causes for these deaths, Drs. R. A. Bielinski, W. S. Harris, A. C. Jain, and M. M. Abdul-Monem of the University of Illinois studied the cardiovascular effects in dogs and cats of inhaling various concentrations of Freon-12 for varying periods of time.

The effects of Freon-12 were dose-dependent and, in dogs, the gas caused substantial reductions in heart-muscle contractility, aortic blood pressure, and blood vessel tone. In cats, Freon-12 reduced heart output in addition to these other changes, which were also more pronounced than in dogs. The gas did not produce disturbances in heart rate or rhythm in either species.

Other study results suggested that Freon-12 is directly toxic to heart and vascular muscle. The investigators conclude that the potent hypotensive and heart-depressant actions of Freon may be important factors in aerosol propellant deaths in man. The research was supported by NHLI.

## ARTERIOSCLEROSIS

Mucopolysaccharides, important constitutents of the connective tissue "cement" that holds together the cellular components of organs and tissues, can form insoluble complexes in the blood vessel wall with lipid-laden liproteins from the blood, according to Dr. Gerald S. Berenson and co-workers at the Louisiana State University School of Medicine.

Employing a technique that enabled them to extract these complexes without causing dissociation of their components, the investigators found that mucopolysaccharides form complexes most readily with low-density lipoproteins and, to a lesser extent, with

very-low-density lipoproteins.

Low-density lipoproteins are the chief transport vehicles for cholesterol in the blood and very-low-density lipoproteins are the chief carriers of triglycerides (neutral fat). Unless combined with lipoproteins, these fatty substances would otherwise be insoluble in plasma.

The NHLI-supported investigators believe that these blood lipids again become insoluble when their carrier lipoproteins form complexes with mucopolysaccharides in the blood-vessel wall and that the formation of these complexes may be a principal mechanism underlying the development of fatty streaks that are thought to be the earliest lesions of atherosclerosis.

## Saphenous Vein Bypass Grafts

In recent years, surgeons have achieved excellent results in removing atherosclerotic deposits or clots obstructing bloodflow in the femoral arteries of the lower abdomen and thigh or else replacing or bypassing the obstructed segment with artificial blood vessel grafts. The result has been relief of much disability and the salvage of thousands of limbs threatened by gangrene.

Surgical experience with the smaller branches of these arteries--mainly the popliteal arteries of the lower part of the leg--has been less extensive. The smaller size of these vessels makes the use of artificial blood vessels inadvisable, because such grafts tend to become blocked by blood clots, often shortly after installation. The use of saphenous vein segments for this purpose appears to avoid this problem.

Drs. Frederick A. Reichle and R. Robert Tyson, of the Temple University Health Sciences Center, Philadelphia, report excellent long-term results employing saphenous vein grafts to bypass obstructions in the popliteal arteries. Of 88 patients studied--all with gangrene, leg ulcers, ischemic leg pain at rest, or other severe symptoms of popliteal obstruction--78 underwent bypass surgery. Functional bypass grafts were achieved in 59, with salvage of limbs in 51. There was one operative death.

Followup of survivors for periods up to 8 years shows that most of the initially successful grafts have remained functional, indicating that the use of saphenous vein bypass grafts allows successful long-term restoration of blood flow in ischemic lower extremities previously considered inoperable. These research studies were supported by NHLI.

## "The Average American Diet" and Atherosclerosis

Rhesus monkeys fed an "average American diet" over a 2-year period developed excessive blood cholesterol levels and atherosclerosis much more extensive and severe than did monkeys fed a "prudent diet" for the same length of time, according to Dr. Robert Wissler and co-workers at the University of Chicago.

Both diets contained many ingredients commonly found on U. S. tables, but the prudent diet contained fewer daily calories, less total fat and cholesterol, and a higher proportion of fat calories in the form of unsaturated fatty acids.

The serum cholesterol levels of monkeys fed the average American diet averaged 540 mg/100 ml, compared with only 219 in monkeys on the prudent diet. At autopsy, monkeys fed the average American diet had atherosclerotic deposits covering 46% of the aortic inner lining (versus 7% in monkeys on the prudent diet), and the extent and severity of atherosclerotic deposits in their coronary arteries was substantially higher.

These results tend to substantiate the contention of numerous scientists who hold that the typical American diet--high in cholesterol, total fat, and saturated fatty acids--is largely responsible for our very high morbidity and mortality rates from coronary heart disease. The results further suggest that coronary heart disease morbidity and mortality might be reduced in the U. S. population through voluntary dietary changes calculated to reduce total fat and cholesterol intake and to substitute, where practicable, unsaturated for saturated fat.

In another study, Drs. Hugh B. Lofland and co-workers, of the Bowman Gray School of Medicine, Winston Salem, N.C., have found that certain squirrel monkeys do not develop elevated blood cholesterol levels despite diets very high in this sterol, whereas other monkeys of this breed develop severe hypercholesterolemia when fed the same diet.

Seeking mechanisms possibly responsible for these differences, the investigators compared cholesterol absorption, production by the body, turnover, and excretion in the two contrasting groups, both fed the same high-cholesterol diet.

The animals that did not develop hypercholesterolemia exhibited more rapid rates of cholesterol turnover and their output of bile acids (the principal pathway for cholesterol excretion) rose more rapidly and to higher levels than in those that developed excessive blood cholesterol levels. Cholesterol absorption and synthesis were similar in both groups.

The investigators conclude that, in this species, blood cholesterol levels are regulated primarily through mechanisms controlling the breakdown of cholesterol or its precursor substances. Both studies were funded by NHLI.

## Estrogens and Atherosclerosis

Female sex hormones (estrogens) appear to protect the blood vessel wall against the harmful "wear and tear" usually resulting from high blood pressure, according to Dr. Harvey Wolinsky, of the Albert Einstein College of Medicine, Bronx, New York.

Estrogens secreted by females during their reproductive years appear to confer protection against the development of coronary heart disease, so that women enjoy a 10-20 year grace period before atherosclerosis begins to attack their blood vessels with the ferocity reserved for men of comparable ages.

It has been assumed that this protection lay chiefly in the effects of estrogens on blood lipids. Estrogens do not dramatically reduce total plasma levels of cholesterol and other fatty substances; they do shift the distribution of these lipids among the lipoprotein classes, so that a greater proportion of plasma cholesterol is carried by the non-atherogenic high-density lipoproteins.

The present NHLI-funded study offers still another possible explanation for the protection afforded by estrogens against atherosclerosis. In this study mice with induced hypertension over an 8-week period typically developed increased thickness of the blood-vessel wall and other degenerative changes thought to favor the development of atherosclerotic deposits. In contrast, extrogen-treated mice, despite similar elevations in blood-pressure levels and in calculated tension and stress on the blood-vessel wall, did not develop these changes, their blood-vessel status remaining virtually the same as that of mice with normal blood pressure used for experimental controls.

Hypertension, especially when it co-exists with elevated blood lipid levels, dramatically increases susceptibility to atherosclerosis, apparently by creating conditions favorable to deposit of plasma lipids in the blood-vessel wall. How estrogens protect the blood vessel against the degenerative changes usually resulting from hypertension is not known; but the study does suggest that the importance of these hormones in atherosclerosis may go beyond their known physiological effects.

## Partial Intestinal Bypass Operations

Over the past 8 years, Drs. Richard B. Moore, Ivan D. Frantz, Richard L. Varco, and Henry Buchwald, of the University of Minnesota, have performed partial intestinal bypass operations in 100 patients, some with gross elevations of blood cholesterol, others with elevated blood triglycerides, and still others with excessive blood levels of both lipid fractions. The research is funded by NHLI.

The operation reduces the absorption of dietary lipid from the gut and may also speed excretion of bile acids and other products of lipid metabolism that might otherwise be recycled by the intestine.

During this period, the procedure has produced reductions in blood cholesterol levels ranging from 31 to nearly 60 percent and triglyceride reductions ranging from 40 to 60 percent.

There was only 1 in-hospital death following the procedure and other complications have been minimal: chiefly diarrhea, which has proved transient in most instances.

The investigators conclude that partial intestinal bypass is a low-risk procedure usually effective in controlling refractory blood-lipid abnormalities, some of which are strongly associated with premature development of atherosclerosis.

## PULMONARY DISEASES

Dr. Charles Mittman and co-workers, of the City of Hope Medical Center, Duarte, California, report that a heritable intermediate deficiency of the blood enzyme alpha-1-antitrypsin predisposes affected individuals to the development of chronic obstructive lung disease. The deficiency apparently accentuates the harmful effects of external irritants, especially cigarette smoke. The NHLI-supported study results further suggest that some chronic lung disease might be prevented by identifying persons with a moderate deficiency of this enzyme before they develop symptoms and by counselling them to avoid cigarette smoking or other lung irritants.

Persons who inherit the defective gene responsible for this deficiency from both parents are relatively rare; most of them develop chronic lung disease before age 40 whether or not they smoke. Persons who inherit the defective gene from only one parent are less susceptible, though still at substantially higher risk than normal individuals. This group may comprise upwards of 5% of the general population.

In studies on 61 presumably healthy relatives of 29 patients with known chronic obstructive lung disease and antitrypsin deficiency, 33 were found to have intermediate alpha-1-antitrypsin deficiency. Dr. Mittman and co-workers found that almost half of the 12 relatives who demonstrated the intermediate deficiency and were also smokers had evidence of definite airway obstruction. Only one subject of the 21 non-smokers had evidence of airway

obstruction.

The investigators suggest that individuals with a family history of chronic obstructive lung disease be screened for alpha-1-antitrypsin deficiency, since early detection of the trait, coupled with avoidance of cigarettes and other lung irritants, may enable many heterozygous individuals to escape the consequences of this genetic predisposition.

## THROMBOSIS AND HEMORRHAGIC DISEASES

Clots in the coronary arteries (the principal cause of acute heart attacks) and injured heart-muscle tissues "downstream" from such an arterial obstruction attract swarms of blood platelets. These may become trapped in smaller branches and collateral vessels within and adjacent to the blood-deprived area, further reducing the amount of blood that can get through, according to Dr. Timothy J. Regan and co-workers of the College of Medicine of New Jersey, Newark.

In studies supported by NHLI, dogs were infused with platelets tagged with chromium-51, a radioactive tracer. Then a coronary thrombus was induced with an electrode-tipped catheter. The animals were sacrificed one hour later and the distribution of platelets in blood and in blood-deprived (ischemic) and non-ischemic areas of heart muscle was determined by scintillation counting. The concentration of platelets at the site of arterial obstruction was 150 times that of blood, and their concentration in and adjacent to blood-deprived areas of heart muscle was 10 times that of non-ischemic areas.

In subsequent experiments, dissolution of coronary thrombi with the clot dissolving agent urokinase substantially reduced platelet concentrations in the ischemic areas. The investigators feel that fibrinolytic agents, by dispersing the platelet swarms in ischemic tissues, may improve its blood supply even before the large vessel clot has been broken down by the drug.

### Drugs and Pulmonary Hypertension

Drs. C. B. Rosoff and E. W. Salzman of Harvard Medical School, report that release of the vasoactive substance serotonin by blood platelets may be an important factor in the constriction of lung blood vessels that commonly occurs after pulmonary embolism. This constriction may further reduce a pulmonary blood supply already compromised by clots obstructing one or more lung arteries, and so worsen the patient's prospects for recovery.

Their studies in animals indicate that agents which inhibit the accumulation and concentration of serotonin by blood platelets (the antihypertensive drug reserpine is such an agent) or which block platelet release of this amine (as aspirin apparently does) protect against this pulmonary blood-vessel constriction and substantially improve survival after pulmonary embolism.

For example, rabbits receiving large doses of aspirin prior to experimental induction of pulmonary embolism did not develop pulmonary hypertension and all survived. In contrast, untreated rabbits subjected to the same embolization procedure all developed severe pulmonary hypertension and 45 percent succumbed.

## Platelet Aggregation Inhibitor

Cyproheptadine (Merck, Sharp & Dohme) an antihistamine and serotonin antagonist used in the treatment of various skin rashes and certain allergies, is a potent inhibitor of blood platelet aggregation, according to a report by Drs. Barbara Goldman, L. M. Aledorf, Elena Puszkin, and Lewis Burrows, of the Mount Sinai School of Medicine, New York City.

In the test tube, concentrations of 2.5-15 milligrams per 100 milliliters abolished the platelet aggregation normally elicited by the clotting factor thrombin, connective tissue, the hormone epinephrine, and the intracellular compound adenosine diphosphate all of which may participate in the formation of platelet thrombi in the body.

The results indicated that cyproheptadine blocked both the uptake and release of serotonin by platelets, and this action appeared responsible for its effects on platelet aggregation.

This property of cyproheptadine, coupled with its low toxicity, suggests various clinical applications. Platelet aggregation in transplanted organs is one of the early phases of the rejection response, and there is both laboratory and clinical evidence of the drug's value in protecting threatened kidney transplants. Platelet thrombi are also suspected in certain clotting complications of atherosclerosis and following the insertion of artificial heart valves and related prostheses.

## BIOENGINEERING

Dr. P. N. Sawyer and co-workers, of the Downstate Medical Center, Brooklyn, and the Chemical Automation Corporation, report their results with a mechanical ventricle powered by artificial "muscles" of nitinol, a nickel-titanium-cobalt alloy. Strands of this alloy, when activated by electricity, contract in a manner analogous to muscle fibers.

The investigators fabricated flexible heart chambers from ethylene vinyl acetate. Strands of nitinol, anchored to the exterior of the chambers, were energized by an integrated electrical circuit so that their contraction imparted a "wringing" action very similar to that occurring during normal left-ventricular contraction.

The artificial ventricle could be stimulated to "beat" 12-15 times per minute, pumping 25-25 cubic centimeters of water per stroke against pressures as high as 160 centimeters of water.

Artificial contractile elements developed earlier had such low efficiencies that the application of artificial muscles to blood pumps appeared all but impossible. The performance of the pump developed during these NHLI-funded studies does not approach what would be required of a total heart replacement, but the results do suggest the potential feasibility of this approach.

## Measurement of Vascular Bloodflow

Nuclear magnetic resonance may provide a simple, dependable, non-invasive means of measuring venous or arterial bloodflow, according to Dr. Robert Bowman and Mr. V. Kudravcev, of the NHLI Laboratory of Technical Development, who have been working on the technique in collaboration with scientists at the University of Wisconsin and the Badger Meter Company, Milwaukee. The prototype devices developed thus far are chiefly suitable for monitoring bloodflow in the limbs, but the scientists feel that the technique may also prove applicable to other vascular beds, such as the cerebral blood vessels.

The technique capitalizes on the fact that hydrogen ions, which are always present in the blood, become transiently magnetized when they pass through an externally imposed magnetic field. In the devices developed thus far, this is done with a permanent magnet installed in the upper portion of a sleeve fitting around the limb in which flow is being measured.

Slightly "downstream" from the permanent magnet is a radiofrequency magnetic coil--also built into the sleeve--that detects the previously magnetized hydrogen ions and generates a nuclear magnetic frequency signal. When adjusted for distance between components and for the strength and size of the upstream magnetic field, this signal is proportional to the velocity and volume of bloodflow.

The detector coil is sensitive only to magnetized hydrogen in moving fluid and is not affected by static tissues between it and vessels in which flow is being monitored. It can measure steady or pulsing flows as low as 20 milliliters per minute.

The investigators feel that the technique, with continued development, may have important applications in screening for various circulatory disorders and in patient monitoring.

## Measurement of Blood-Oxygen Levels

An electro-optical device called the Optisat LTD-1500, which provides continuous measurement of blood-oxygen levels during surgical procedures under heart-lung bypass or respiratory-assist procedures employing blood oxygenators, has been developed by Dr. Gerald Vurek, of the NHLI Laboratory of Technical Development, and Mr. Walter S. Friauf, of the Division of Research Services (DRS).

The device was selected as one of the 100 most significant new technical products of the year in an international competition

sponsored by Industrial Research, Inc.

As blood is returned from the oxygenator to the patient, it is routed through a segment of clear plastic tubing in the Optisat, where it is illuminated alternately with red and infrared light. The hemoglobin of the red blood cells, when combined with oxygen, absorbs visible red light more avidly than does unoxygenated hemoglobin. Thus the greater the oxygen saturation of blood, the greater its absorption of visible red light.

Light absorption is monitored by a photoelectric cell, which generates an electrical signal roughly proportional to blood-oxygen saturation. Infrared illumination is used to compensate for variations in light absorption resulting from changes in bloodflow or hematocrit. Both oxyhemoglobin and hemoglobin absorb infrared about equally. Thus, by taking the ratio of the infrared signal to the red signal, blood oxygen saturation can be computed independently of these variables.

The Optisat provides immediate continuous measurements without bloodflow obstruction or withdrawal of blood samples. The information is displayed visually in analog or digital form and can be recorded, if desired.

## Intra-Aortic Balloon Pump

An intra-aortic balloon pump for providing temporary pumping assistance to the failing left ventricle has been employed in 39 patients with cardiogenic shock by Drs. W. B. Dunkman, M. J. Buckley, R. C. Leinbach, E. D. Mundth, A. R. Kantrowitz, and W. Gerald Austin, of Massachusetts General Hospital, Boston.

Cardiogenic shock, the complete circulatory collapse that sometimes occurs in the wake of severe heart attacks, appears to stem chiefly from failure of the heart as a pump (due to massive injury sustained during the attack) coupled with loss of normal blood-vessel tone. The result is usually a catastrophic fall in heart output and blood pressure that currently carries mortality rates approaching 100%.

The intra-aortic balloon pump is a mechanical device designed to provide temporary pumping assistance to a failing left ventricle. Threaded into the aorta on the end of a catheter and synchronized with the patient's heartbeat, the balloon is inflated while the patient's heart is refilling between beats, then deflated just before the patient's heart contracts. Inflation of the the balloon raises arterial blood pressure and improves the blood supply to the heart muscle itself; deflation just before heart contraction enables the injured heart to work against a reduced pressure in pumping blood.

The cardiogenic shock patients were treated with intra-aortic balloon pumping only after an initial trial of conventional medical therapy had failed to improve their clinical status. The delay in application of balloon pumping averaged 14 hours in 31 patients and

3-9 days in the others.

The circulatory assistance for 24-48 hours substantially improved circulatory dynamics and reversed the shock syndrome in 31 patients; but in most this improvement lasted only as long as the circulatory assistance was continued, and only 5 of 26 patients treated solely with balloon pumping survived. In 13 others, who were conceded no chance of surviving with balloon pumping alone, emergency surgery was performed to improve the blood supply to the heart muscle or to remove hopelessly damaged heart tissue, or both, with circulatory assistance continued throughout the preoperative and immediate postoperative period. Five of the 13 survived and were doing well from 2-10 months later. NHLI supported the research.

The authors conclude that intra-aortic balloon pumping, although not so effective as one might wish in reducing overall mortality from cardiogenic shock, nevertheless saves some who would almost certainly die otherwise and can enable others to undergo potentially life-saving surgery.

## STROKE

A new technique for production of better X rays of blood circulation in the brain has been developed by Dr. Fletcher McDowell and co-workers at the Cerebrovascular Research Center at Cornell University Medical College, supported by the National Institute of Neurological Diseases and Stroke (NINDS).

The technique, called serial circular angiotomography, helps to solve the problem of depth perception, inherent in any X ray of the brain, by focusing the picture on a specific section or plane within the head. Although tomography is not new, the Cornell workers have refined it to the point where they are able to get diagnostic information not obtainable from conventional films.

### Diagnosis by Radioisotopes

Radioisotopic brain scanning also is being improved at the NINDS Bethesda laboratories. This technique entails injection or inhalation of radioisotopes instead of dye injection, as in X ray, and production of "pictures" by radiation detectors. It has suffered in the past from the problem of depth perception.

NINDS scientists, Giovanni Di Chiro, Florio Miraldi, and Eugene Yon, working in collaboration with engineers at Case Western Reserve University, have designed and built a scanner which they hope will get results similar to tomographic X ray. Their device has been installed at NIH, and is undergoing tests. If successful, it will be an important innovation in neuroradiology, since scanning offers many advantages over X ray, including complete safety from reaction to the dye, shock, or accidental damage to the vascular system; there is no discomfort to the patient or need to admit him to a hospital, less radiation is involved and there is less expense.

## Treatment of Aneurysms

Success with a novel advance to treatment of aneurysms (balloon-like arterial protrusions, which may burst, causing hemorrhage into the brain) was reported this year by NINDS grantee John F. Alksne at the Medical College of Virginia. The method entails use of a tube-shaped magnetic probe through which a needle is passed for injecting powdered iron into the aneurysm.

The magnetic probe, which is put through a burr hole in the skull and moved into a position abutting the aneurysm, holds the iron particles in place for three to five days while a clot forms around them, thus creating a "plug." Eventually, the clot and aneurysm are replaced with scar tissue. Experience with the technique has varied, depending on the condition of the patients and location of the aneurysms, but in one group of 15 patients, results were considered very encouraging in that ten were able to return to work.

## Blood Deprivation in Brain Tissue

The horizons of intracranial vascular surgery were extended in 1971 by scientists at NINDS who demonstrated, in monkeys, that brain tissue is more resistant to damage from blood deprivation than was previously thought. The question takes on increased importance with the development of microsurgical techniques for use within the brain.

Data, supplied by Drs. Robert Crowell, Yngve Olsson, Igor Klatzo, and Ayub Ommaya, based on experimental blockage of the middle cerebral artery, offer hope that severe brain damage might be avoided in many patients if the surgeons could operate soon enough after an occlusion. Closing off the arteries with clips, the scientists found that 1 to 2-hour clipping caused either no deficit or only mild neurological deficit when the monkeys were examined 1 to 3 days later. Four-hour clipping caused mild to moderate damage, and only clipping for 6 hours produced severe infarction (local brain cell death) similar to that caused by permanent occlusion.

## Brain Blood Vessel Therapy

A new vasodilator (one of a group of drugs which relax brain blood vessels and allow them to open to their fullest diameter) was reported favorably by Dr. John Stirling Meyer at Baylor College of Medicine in Houston. The drug, hexobendine, was found to increase hemispheric blood flow and metabolism in 18 patients with cerebral infarction, and caused no serious side effects.

Urokinase, a clot-dissolving agent, is undergoing trials in stroke patients at Washington University in St. Louis. The scientists there, headed by Dr. Anthony Fletcher, have developed a powerful new technique--plasma fibrinogen chromatography--for measuring substances entering into the process of clotting.

The relative proportions of these substances--fibrinogen complexes and derivatives--are a guide to abnormal clotting

tendencies, and give the scientists an indication of how to plan treatment. Patients in whom a brisk, spontaneous response of the clotting system is detected are assumed to require either no therapy or only minimal clot-dissolving therapy, while others, in whom the technique indicated a poor response, are treated. Urokinase is extremely expensive and causes toxic reactions and bleeding when given in large doses, thus a reliable means of monitoring its action and keeping the dose down to the lowest level necessary would be a welcome advance.

## Epidemiology of Stroke

Drs. Jack Whisnant, John Fitzgibbons, Leonard Kurland, and George Sayre at the University of Minnesota made an important contribution in 1971 toward solving one of the problems in stroke epidemiology--lack of a reliable statistical baseline for comparison with various populations and time periods--in a study of records from the Mayo Clinic in Rochester, Minn. Epidemiologists have been troubled for many years by inadequate reporting on death certificates, changing diagnostic fashions, the problem of getting accurate data on mild cases seen at home, and various other factors rendering many of the earlier stroke incidence and prevalence studies suspect. The findings are unusually reliable because they used data from the Mayo Clinic, which has high medical standards combined with an unusually good record-keeping system, and because they included a large number of mild, non-hospitalized cases. Highlights of the NINDS-funded study were:

> The finding of an incidence rate of 194 per 100,000 per year. This is higher than that found in most previous studies, and if applied to the whole U.S. population would give a figure of about 400,000 new strokes per year.
>
> The finding that 9.4 percent of the strokes were due to cerebral hemorrhage. This has been one of the most misused terms in medicine, as illustrated by a comparison of figures from Vital Statistics of the United States from 1905 through 1965: the ratio of cerebral hemorrhage to cerebral infarction was shown as 80 to 1 in 1920, and 2.5 to 1 in 1955. This distinction is critical because of the important differences in treatment for each of these disorders.
>
> The finding that among survivors of first strokes, nearly twice as many die from heart disease as from a subsequent stroke. This is much higher than figures reported previously.

## DENTAL DISEASES

Disease prevention is the most promising route to oral health, and this year the National Institute of Dental Research (NIDR) reports encouraging progress toward the development of new preventives, as well as improved restorative materials and treatments.

## ADVANCES FOR DENTAL PRACTICE

One treatment with a plastic sealant can protect the pits and grooves on the tooth's griding surface for two years, according to reports from Dr. Michael Buonocore, and NIDR grantee at the Eastman Dental Center, Rochester, N.Y.

The sealant, one of several now on the market, is painted onto cleaned and slightly-etched chewing surfaces of teeth. Exposure for a few seconds to a long-wave ultraviolet light hardens the plastic paint.

The application of the sealant is simple, safe, and painless. Large-scale clinical trials are underway to determine if sealants are practical as a public health measure.

Carboxylate cements offer promise for several uses. Tests made by NIDR-supported scientists with one such commercially-available cement have verified that it adheres chemically to tooth enamel and thus might serve as a sealant.

Because of its strong adhesion and its compatibility with the tooth's sensitive pulp, the cement is also a prime candidate for securing fillings, gold inlays and crowns to teeth.

Once the material is perfected--it must be strengthened and its adhesion to metal improved--it could replace orthodontic bands. The tiny brackets used to hold orthodontic wires could be attached directly to the teeth through use of the cement.

Laboratory and clinical tests of this promising material are continuing by the grant-supported team of Dr. R. W. Phillips, M. L. Swartz, and B. Rhodes at the Indiana University.

Basic studies of amalgam, the traditional siver dental filling, have led directly to a new and stronger material. A University of Virginia metallurgist, Dr. Lewis B. Johnson, Jr., discovered that substituting a little gold for some of the silver in the mixture makes a better amalgam. Laboratory tests indicate that fillings of the new amalgam should be more durable, and accordingly, clinical trials are now being planned.

A new method of bonding plastic teeth to plastic denture bases has been developed by Drs. N. W. Rupp, R. L. Bowen, and G. C. Paffenbarger, NIDR grantees with the American Dental Association's Research Unit at the National Bureau of Standards. The new method is more effective, yet simpler than the current practice of cutting grooves into artificial teeth to get mechanical retention and then

adding a weak cement.

Tests developed by NIDR grantees to determine safety of dental and medical materials show that sterilizing plastic tubing and other reusable medical supplies by fumigation can produce a poisonous liquid, which can quickly penetrate skin. Initial removal by the manufacturer of any poison following fumigation of these products cannot prevent its formation later. University of Tennessee toxicologists, Drs. W. H. Lawrence, J. E. Turner, and J. Autian, therefore have recommended that hospitals check plastic items after re-sterilization. Devised principally to test the toxicity of biomaterials for dental restorations, the test system is beginning to be used to check new alloys, plastics, and devices as they are developed.

## PAIN CONTROL

Meprobamate, a drug with tranquilizing and muscle-relaxing properties, relieves a painful facial joint syndrome, according to Drs. David M. Laskin and Charles S. Greene of the University of Illinois Medical Center. The pain is believed to stem from muscle spasms, often caused by tension-induced teeth clenching. Evidence suggests that the tranquilizing effects of the drug are more helpful than its ability to relax muscles, probably because subjective tensions are an important cause. Psychological studies showed that many of the individuals affected by the syndrome are either over-conscientious, dominant women, or persons having tendencies toward psychosomatic problems.

Anesthesiologists, testing medications used prior to anesthesia to reduce anxiety and fear of oral surgery, found that certain drugs can reduce pain from pressure of manipulations but increase sensitivity to heat pain. Drs. G. L. Freedman and G. D. Allen of the University of Washington conclude that both the sedative promethazine and the pain killer meperidine supplement effects of a local anesthetic in reducing pressure and dental pain. In types of surgery where little manipulation is required, the sedative pentobarbital should suffice to allay anxiety.

Chewing on ice or drinking hot coffee with ice cream, in time, can make teeth crack, mechanical engineers at the University of Utah, supported by NIDR, report. Drs. W. S. Brown and H. R. Jacobs, and R. E. Thompson, found that expansion from heat and sudden contraction from cooling can crack teeth because enamel and the dentin layer beneath it expand and contract at different rates. Furthermore, enamel is a poor conductor, so that when it is cooled suddenly it cannot contract because the dentin beneath it has not yet cooled and contracted. The resultant thermal stress can crack teeth.

These findings may help explain why heat and cold cause dental pain. With cold, contracting enamel may squeeze dentin until it presses against the sensitive nerve endings in the pulp. Expansion from heat could also make dentin close in on the pulp and trigger pain.

## PERIODONTAL DISEASE

Extraction of upper molars should be avoided in treating periodontosis, a form of periodontal disease affecting adolescents and young adults that progresses rapidly and is marked by severe bone loss. Drs. P. N. Baer, NIDR, and F. G. Everett, University of Oregon in Portland, make this recommendation. They compared long-term changes in adjacent bone in patients whose upper molars were extracted because of the disease, with bone loss in others who had been given extensive, time-consuming treatment. In a relatively short time after extractions, bone loss was so severe that adjacent teeth were endangered. Early diagnosis and treatment are imperative to preserve teeth and the integrity of the entire jaw.

If bone loss from periodontal disease is extensive, too little bone may be left to support dentures, especially for the lower jaw. Ways to induce bone formation are being sought to help patients with this result of periodontal disease, as well as accident victims with shattered bone and cancer patients who have undergone bone removal. Dr. M. Urist of the University of California discovered that demineralized bone triggers new bone formation. Another grantee, Dr. H. Wells of Boston University, successfully used similarly-treated dog bone to fill defects in dog jaws, and rat bone to bridge large gaps in legs of rats. The grafts were accepted and in two months new bone replaced the graft, repairing the defects.

## DENTAL PLAQUE

Billions of organisms collect on the teeth around and underneath the gum line of susceptible persons, forming a mat called dental plaque. Many of the bacteria in plaque have the capability of fermenting sugar into acids which cause tooth decay. Other bacterial products are thought to trigger gum inflammation and to cause most periodontal disease in which soft tissues and bone supporting the teeth are attacked.

Research is now finding that antimicrobial agents can control dental plaque diseases. The effectiveness of one antiseptic already is being studied on human patients in Europe. Other tests made at the NIDR by Dr. J. M. Tanzer and colleagues have shown that chlorhexidine disrupts plaque growing either on wires in cultures or on accessible parts of hamster teeth. Although Europeans have used the antiseptic externally for years, its flavor poses some problems in a mouthwash. Nevertheless, the antiseptic is regarded as a forerunner in the search for anti-plaque agents.

Dr. W. J. Loeshe and associates at the University of Michigan report that a paste of kanamycin sulfate can control severe gingivitis, on the basis of studies of handicapped, institutionalized people. Still another antibiotic (vancomycin), tested in hamsters by NIDR scientists, Drs. H. R. Englander and P. H. Keyes, controls not only plaque formation and severe gum inflammation, but also rampant tooth decay.

Though both drugs appear promising and may be used to control

severe dental disease, no single "miracle drug" is expected. Dental scientists believe regular tooth brushing and use of dental floss continue to be the prerequisite techniques for those able to care for themselves.

## TOOTH DECAY

To find other elements beside fluoride that protect against decay, epidemiologists are studying isolated peoples who have low caries rates For unknown reasons, children in one isolated town in South America have less tooth decay than children from a nearby village, according to NIDR grantees from Harvard University and the University of Antioquia in Colombia. In fact, from what is known about tooth decay, the existing conditions seem to favor the town with the higher--not the lower--caries levels. To explain this paradox, scientists are searching for some inapparent hereditary factor or some trace substance in the soil, water, or foods.

In another primitive people, Guatemalan Indians, less tooth decay was associated with mineralized plaque. Drs. L. V. Sutfin, E. A. Sweeney, and W. Ascoli of the Massachusetts Institute of Technology, suggest that the high calcium and phosphorus levels fround in the Indians' tortilla diet, but not in western white bread, may not only harden the plaque but also replace minerals usually removed in the decay process.

Phosphates have long been known to reduce decay in laboratory animals. For human tests, scientists have decided to put them into chewing gum or cookies rather than into water because evidence suggests that phosphates work through some local action on teeth rather than systemically. Drs. J. M. Navia and F. S. Tanzer at the University of Alabama proved this mode of action in studies on paired rats with surgically-joined intestines. Although only one of each pair chewed the phosphate-containing diet, both would obtain a systemic benefit from digested food. The fact that the rats which chewed the phosphate developed less decay than did their artificial twins is therefore significant.

## BACTERIAL INTERACTIONS

To find new ways to stop plaque formation and thereby prevent decay and periodontal disease, dental researchers are studying the complex interactions in the highly organized plaque ecological system. Dr. R. J. Gibbons and associates at Forsyth Dental Center in Boston are examining mechanisms of bacterial adhesion on the assumption that microbes must attach to a tissue before they can attack it. Studies with human volunteers confirmed the team's earlier laboratory findings that some kinds of microbes usually are found on tooth enamel, while others favor the gums, mucous membranes or the tongue. Still others cling only to established plaque, and some types need to react with polymers in saliva to adhere.

University of Oregon grantees find that a prevalent microbe (Neisseria), which does not cause decay by itself, uses starch to make a substance (amylopectin) on which other bacteria grow and

produce decay-causing acids. This finding of Drs. R. B. Parker and H. R. Creamer helps explain why some people who eat little of the sugar which leads to most decay can still develop cavities.

Once the gum-tooth barrier is broken and plaque and calculus push the gums away from the teeth, organisms can flourish in the pockets formed, and attack both the tooth roots and the tooth-supporting soft tissues and bone. Drs. J. Kelstrup and R. J. Gibbons of Forsyth Dental Center, conducted experiments in which germfree rats were infected with one strain of streptococcus, a culprit in tooth decay. Severe loss of jawbone as well as decayed tooth resulted.

To control both periodontal disease and root surface decay, plaque preventives must be developed. While crown decay can be repaired if treated early enough, it is difficult to fill hard-to-reach tooth roots. Another Forsyth microbiologist, Dr. H. V. Jordan, reports that Actinomyces, a filament-like bacterium suspected in periodontal problems, may also be a chief culprit in root decay. He found the microorganism in root cavities of human teeth lost due to severe periodontal disease.

Two natural defenses against microbes have been found in saliva by Drs. I. L. Dogon and B. H. Amdur, also from Forsyth Dental Center. One chemical system can defend against oxygen-requiring organisms in plaque on the tooth crowns, and the second system can fight oxygen-shunning microbes dwelling deep in periodontal pockets.

## SYSTEMIC FACTORS IN PERIODONTAL DISEASE

Human cells contain destructive enzymes, needed for normal breakdown and repair functions of the body, which can cause disease if too many are manufactured. Recently Dr. J. F. Goggins, L. C. Billups, and P. F. Rothberg of the NIDR found that one type of cell, the fibroblast, contains small amounts of hyaluronidase. This enzyme breaks down hyaluronic acid, an important component of the material surrounding periodontal fibers. In gum disease excess amounts of this enzyme may come from microbes, fibroblasts, or from macrophages, another cell type in which Dr. Goggins has found hyaluronidase. Ordinarily, macrophages are useful scavengers which congregate at infection and inflammation sites, including diseased gums.

### Inflammation and the Immune System

Other NIDR investigators believe that, with time, people tend to become sensitized to bacterial products in the mouth. When these products get into gum tissues, they cause a local inflammation which possibly leads to periodontal disease. Drs. J. E. Horton, G. D. Gordon, S. Leikin and J. J. Oppenheim found that material taken from gum pockets of patients with moderate periodontal disease has practically no stimulating effect on white blood cells from umbilical cords of infants, who are rarely sensitized to foreign proteins. However, in adult blood, certain white cells (lymphocytes), which are active in inflammation, were stimulated by both saliva and pocket material. Such stimulation is accepted by immunologists as evidence of previous sensitization.

Another white blood cell, the monocyte, also takes part in various immune reactions. Normally, monocytes circulate in the blood, but they migrate out of a vessel and pass through tissue to destroy invading bacteria when summoned by endotoxins and antigens. Drs. M. Hausman, R. Snyderman, and S. E. Mergenhagen of the NIDR discovered that endotoxins derived from the walls of dead bacteria trigger the formation of two chemical messengers which attract monocytes. One is light-weight and heat-resistant; the other, a heavy molecule, is inactivated by heat.

The light-weight chemical attractant does more than helpfully summon white blood cells. It can contribute harmfully to inflammation by making blood vessels leaky, and takes part in other immune reactions, such as smooth muscle contraction and shock. Dr. R. Snyderman and others at NIDR have shown that this messenger molecule can stimulate the same immune reactions in living mice that it triggers in cell cultures.

## IMMUNOLOGY AND TRANSPLANTS

The immunologic protest by which the body rejects most grafts as foreign material is the greatest barrier to tooth and organ transplants. Previous studies of mice show that the chief determinant of tissue compatibility for transplants or grafting is a specific part of the cell wall--an antigen. The structure of this antigen is controlled by a gene; therefore, tissues from mice with the same gene are relatively suitable for grafting.

Two tests developed at NIDR quickly show when this particular antigen is present, and when it has been made soluble. Immunologists think that is this antigen can be detached from cell membranes and made soluble, it may then be used to induce acceptance of foreign tissue, thus contributing to the success of transplants. NIDR scientists, Drs. J. H. Pincus and R. O. Gordon, have succeeded in making a soluble fraction of this antigen from one mouse strain.

Another new, yet simple, test uses cultured white blood cells to determine whether or not they possess significant immunity to specific strains of cancer cells. Some scientists think that one reason cancer is not more widespread is due to a natural immunity. This new way to detect tumor immunity was developed by NIDR's Dr. J. J. Oppenheim and Drs. B. Zbar and H. Rapp of the National Cancer Institute. When guinea pigs are sensitized by inoculating them with certain tumor cells from other animals of the same strain, white cells taken from the abdominal cavity develop a protective response called delayed hypersensitivity. When the tumor cells are added to cultures of these sensitized white cells, the tumor cells are unable to incorporate a substance necessary for growth, tagged to make it radioactive. In this way it is possible to show that enough immunity has developed to control cancerous growth, at least with one variety of cancer.

Dr. Robert Stern of NIDR has modified a chromatographic column (BD cellulose) developed in British Columbia for use in separating neucleic acids of similar molecular size but with different configurations. He and Dr. Robert Friedman of NCI discovered that all cells they examined--even from presumably uninfected chick embryos--contain a viral-like RNA. The role of the RNA is not yet known, but the scientists found that it stimulates production of interferon, a natural antiviral defense in the body. The RNA could also be a latent "oncogene" which after years or generations of inactivity is somehow triggered to reproduce and cause disease

Dr. Stern notes that other substances with a similar structure to that of the natural viral-like RNA also stimilate production of interferon. Therefore, the column could be a tool for quick and inexpensive screening of natural and synthetic substances for potential use as antiviral agents.

## ARTHRITIS AND METABOLIC DISEASES

Important research advances were made during the year against a broad array of arthritic, rheumatic and collagen diseases and many metabolic disorders, including diabetes and other inborn errors of metabolism, kidney disease, nutritional and endocrine disorders, and related subjects.

### ARTHRITIS

The term arthritis refers to a group of diseases characterized by inflammation and impairment of the joints and closely related tissues. It is estimated that 17 million Americans are afflicted with some form of arthritis.

A main target of research by the National Institute of Arthritis and Metabolic Diseases (NIAMD) is rheumatoid arthritis, the most serious and crippling form of arthritis, which affects more than 4,000,000 persons in this country alone. The weight of evidence today suggests that rheumatoid arthritis (RA), may result indirectly from an infectious process. Thus, model diseases known to be caused by microorganisms, with joint lesions comparable to those of RA, are of particular interest.

NIH scientists in Bethesda recently have characterized the clinical, microbiologic, and structural features of an experimental type of arthritis that was induced in swine by injections of Mycoplasma hyorhinis.

Drs. John L. Decker and J. A. Barden described a severe illness which occurred 4 days after intraperitoneal injection and which, by the fifth day, became a frank polyarthritis with swollen joints, toe walking, and limping. By the sixth week all infected animals had pronounced arthritis, which persisted throughout the 8-month period of study, even though it had become progressively more difficult to find living M. hyorhinis organisms in the joints-- an indication that immunologic changes may have been responsible for the long-term pathology.

Of particular interest was the observation that antibody levels in the joint fluid exceeded those in the blood, suggesting local production of antibodies in the joints. It is believed that the findings in this study will shed some light on the cause and development of human rheumatoid arthritis.

## Systemic Lupus Erythematosus

Systemic lupus erythematosus, or SLE, is an insidious, inflammatory connective tissue disease of considerable gravity. Like RA, its cause is as yet unknown. Much evidence, however, points to a disorder of the body's immune mechanism, perhaps tied to an infectious process.

A team of NIAMD scientists, Drs. Alfred Steinberg and Norman Talal, together with investigators in Boston, recently described the frequent finding of antibodies to double-stranded ribonucleic acid (RNA) in the blood of patients with SLE, but rarely in patients with other rheumatoid disorders. Attempts are now being made to understand the specificity and possible significance of these particular antibodies, which may represent a response to viral infection. Various studies have suggested that a combination of genetic, immunologic and viral factors underlies the inflammation and connective tissue degeneration characteristic of SLE.

## Lupus Nephritis

There is no satisfactory therapy for lupus nephritis, a grave kidney disorder associated with SLE. The same scientists, however, who detected antibodies to double-stranded RNA in SLE patients also have shown in prelimary studies that cyclophosphamide, an immunosuppressive drug, may have potential for treatment.

Cyclophosphamide therapy was confined to patients with life-threatening diffuse glomerular disease, as determined by renal biopsy. Significant improvement was observed following 10 weeks of therapy, including reduction of urinary sediment and protein leakage, decrease in antibodies to deoxyribonucleic acid (DNA), disappearance of skin rashes, fever, arthritis, oral ulcers and pleurisy. Despite its severe toxicity, the investigators feel that there may be a place for cyclophosphamide in the treatment of life-threatening lupus nephritis, but only careful patient selection and control can achieve a safe and rational basis for such therapy.

## Gout

An investigator at New York University, Dr. Gerald Weissmann, has demonstrated the precise nature of the molecular biology of acute gouty inflammation and why gout primarily attacks males. His data support the molecular theory of joint inflammation with specific reference to uric acid and gout and also provide circumstantial evidence that the final common process of joint destruction in many arthritic conditions is mediated by leakage of tissue-digesting lysosomal enzymes from cells in the joint or joint fluid.

## Scleroderma

Dr. Alphonse T. Masi of the University of Tennessee, has completed the first epidemiologic morbidity study of scleroderma ever conducted in a large, defined population group. Scleroderma is a chronic disease of unknown cause characterized by fibrosis, rigidity and thickening of the skin and subcutaneous tissues, and frequently involves internal organs, where the functioning tissues atrophy gradually and are replaced by fibrous tissue.

Dr. Masi found the average annual incidence of scleroderma was 2.7 new patients per million population, with rates 3 times higher in females than males for both whites and blacks. Incidence increased steadily with age, peaking at 7.6 per million population in those over age 65. No significant racial differences in incidence were observed.

Furthermore, no socioeconomic variables affecting scleroderma incidence were identified, nor was there epidemiologic evidence of an infectious agent contributing to its cause. The observed age, race, and sex pattern of scleroderma resembled that of adult rheumatoid arthritis.

Dr. Masi's study will afford new perspectives on this disorder and suggest clues to its origin and natural history.

## DIABETES

Recent diabetes research has focused upon the oral anti-diabetic drugs. A long-range study supported by NIAMD has questioned the efficacy of two of the most widely used oral agents. Other investigators have described a novel approach to the management of diabetes, and promising results have been obtained in the use of a high carbohydrate diet as a means of controlling elevated blood glucose levels in patients with mild adult-onset diabetes.

One year ago the University Group Diabetes Program (UGDP), supported by NIAMD, reported that the oral antidiabetic drug tolbutamide (Orinase) is no more effective than diet alone in the treatment of patients with mild, adult-onset diabetes. The same group now has revealed that phenformin (DBI), an antidiabetic drug of the biguanide type, is apparently no more effective than diet alone or diet-and-insulin in prolonging the life of patients with asymptomatic, adult-onset diabetes. Moreover, phenformin-treated patients (as in the case of tolbutamide-treated patients) had a higher death rate from cardiovascular disease than patients on diet alone or on diet-and-insulin.

For these reasons, the use of phenformin has been terminated in the UGDP study, as was the use of tolbutamide. Although these results cannot be regarded as definitive proof of adverse effects, they raise serious questions concerning the long-range benefits of tolbutamide and phenformin therapy, and even concerning the importance of strict regulation of blood glucose levels, heretofore considered an important therapeutic goal in providing longevity for

patients with adult-onset diabetes.

## Progress Toward an "Artificial Pancreas"

The development of an "artificial pancreas," which would be capable of delivering insulin automatically to an insulin-dependent diabetic patient at the moment he needs it and in the exact dose required, would be a significant advance in the management of diabetes. This past year, Dr Samuel P. Bessman of the University of Southern California took the first practical step in this direction by devising a blood sugar sensor capable of continuously monitoring glucose levels in the blood and body fluids.

Research is now geared towards miniaturizing the sensor so that it can be implanted in a patient's body and attached to a small implanted pump containing sufficient insulin to keep the patient supplied for an extended period. The pump would release a small amount of insulin whenever the sensor signals arise in blood sugar level.

Several of the National Institutes of Health are supporting investigations aimed at development of a coating for implantable devices which would make them compatible with blood and solid tissues of the human organism. Until this is accomplished, devices cannot be considered truly implantable, and the envisioned implantable insulin reservoir and blood glucose sensor will have to await future developments.

## Dietary Management of Diabetes

Extreme carbohydrate and calorie restriction formed an essential part of diabetes therapy before the availability of insulin, and varying degrees of carbohydrate restriction are still advocated for the control of diabetes. It has been known since 1935, however, that increased dietary carbohydrate improves glucose tolerance in normal subjects, and several recent studies have suggested that similar results might be obtained in diabetic patients.

Evaluation of the effect of a high carbohydrate diet in patients with mild diabetes has revealed that it lowers fasting blood glucose levels and improves glucose tolerance without altering total insulin secretion.

This study by Drs. Daniel Porte, Jr. and Edwin L. Bierman of the University of Washington, Seattle, has demonstrated an improvement in diabetic control. Such a therapeutic approach warrants further evaluation in mildly diabetic patients with some available insulin, either produced naturally or injected. The results obtained may help develop an optimal diet for patients with mild diabetes who usually are treated with "diet alone."

## New Facility for Diabetes Research

The Southwest Field Studies Section of the NIAMD which revealed that the Pima Indians of Arizona have the highest recorded

prevalence of diabetes in any population group in the world, is continuing its efforts to learn why. Opportunities to study diabetes under carefully controlled conditions have been enhanced by the opening of a Clinical Research Section occupying one floor of the new Phoenix Indian Medical Center.

## OTHER METABOLIC DISEASES

The Hurler's and Hunter's syndromes are similar hereditary disorders of metabolism characterized by skeletal deformities, mental retardation and early death. NIAMD scientists in Bethesda had shown in earlier studies that the excessive intracellular accumulation of mucopolysaccharides characteristic of these disorders is due to a decreased rate of degradation of these substances, rather than to an increased rate of synthesis.

Because this abnormal intracellular accumulation was known to disappear in the presence of human blood in laboratory studies, the therapeutic effects of normal human plasma infusions have been evaluated by Dr. Nicola Di Ferrante of Baylor University. The striking clinical and biochemical improvement observed in patients with Hurler's and Hunter's syndrome who received infusions of normal human plasma suggests that normal plasma may contain factors involved in normal degradation of mucopolysaccharides, and that periodic plasma infusions may modify the course of these fatal disorders. The findings lend hope to the possibility of altering dramatically the progressive downhill course of these diseases when concentrated and purified preparations of the plasma "factors", probably enzymes or enzyme cofactors, become available.

## DIGESTIVE DISEASES

The field of digestive diseases is one of the most challenging and fascinating for both biomedical research investigators and for practicing physicians. A number of significant findings have emerged recently from intensified efforts to improve diagnosis and treatment of gastrointestinal disorders.

### Gallstones

It is estimated that 15 million Americans may suffer from gallstones. Although temporary relief from the distressing symptoms of gallstones can be achieved by administration of a number of agents, permanent relief is attained only by surgical removal of the offending stones. The search for a less drastic method of controlling gallstones has been going on for many years.

The formation of liver bile supersaturated with cholesterol is considered an important first event in the formation of cholesterol gallstones, the most common type. In an earlier study, Dr. Alan D. Hofman of the Mayo Clinic, Rochester, Minnesota, had shown that the proportions in bile of chenodeoxycholic acid, a primary bile acid essential to solubilization of cholesterol, are significantly below normal in patients with cholesterol gallstones.

Dr. Hofman has now reported progressive dissolution of cholesterol gallstones in 4 of 7 patients who were given synthetic chenodeoxycholic acid orally every day for 6 to 22 months. These preliminary findings are so promising that controlled studies on large numbers of patients are being initiated at the Mayo Clinic and at Cedars-Mount Sinai Hospital, Los Angeles.

Liver function and liver structure were unaffected by ingestion of the new agent and the only symptomatic complaint, diarrhea, was controlled by reducing the dosage This is the first report of gallstone dissolution in man, under controlled conditions, using a single agent on a rational basis.

## Gallbladder Disease

NIAMD scientists who had shown that the Pima Indians of Arizona have a rate of gallbladder disease six times higher than that found in Caucasians in Framingham, Mass., are now attempting to associate this high rate with possible predisposing conditions, such as pregnancies, body weight, and diabetes (which also is extraordinarily common among the Pimas).

Their most recent study, a detailed diet survey of Indian women, has indicated that their nutrient intakes do not differ markedly from those of the general United States population. Recent progress in the study of bile acid metabolism and biliary cholesterol secretion has been rapid, and is now directed toward non-surgical approaches to prevention and treatment.

## Lactase Deficiency Syndrome

In 1965 NIAMD-supported scientists demonstrated that milk intolerance due to deficiency of the intestinal enzyme lactase is relatively common in adults. Subsequently, Dr. Theodore M. Bayless of Johns Hopkins University showed that this syndrome is very common among Negro and Oriental adults, suggesting a genetic basis for lactase deficiencies.

Dr. Bayless now has demonstrated the precise mechanism by which lactose, or "milk sugar," causes such symptoms as bloating, cramps, and diarrhea in susceptible individuals. Lactose-induced diarrhea in lactase-deficient subjects results, in large part, from the combination of osmotically induced net fluid secretion by the small intestine and by interference with net fluid absorption in the colon. His findings provide a better understanding of milk intolerance in adults, as well as a rational basis for better management of this widespread disorder.

## Celiac Disease

Celiac sprue is a chronic nutritional and gastrointestinal disorder characterized by fatty diarrhea, intestinal malabsorption, and dietary deficiency symptoms, all of which are usually corrected by excluding the cereal protein gluten from the diet. Although the exact mechanism by which gluten interferes with

absorption is not known, evidence has been accumulating to suggest that an immune response is involved.

Scientists of the NIAMD and the National Cancer Institute, Drs. L. Laster and W. Strober, now have shown that gluten stimulates increased intestinal synthesis of certain antibodies, immunoglobulins, in patients with celiac sprue Thus, the small intestinal mucosa apparently is capable of local synthesis of certain immunoglobulins, and, in patients with celiac sprue, this mechanism apparently responds to exposure to dietary gluten.

## Peptic Ulcer

Prostaglandins, a group of acidic lipids, are widely distributed in mammalian tissues and are highly active in many biological systems. Although prostaglandin $E_1$ is known to be a potent inhibitor of gastric secretion in animals, its use in man has been associated with a number of serious side effects which had prevented therapeutic application.

Dr. Robert A. Levine of the Downstate Medical Center, Brooklyn, now has shown that a derivative of prostaglanding $E_1$, namely prostaglandin $A_1$, significantly inhibits gastric secretion in healthy volunteer subjects and is well tolerated. Thus, prostaglandin $A_1$ may have potential value in the treatment of such disorders as peptic ulcer and gastric mucosal bleeding.

## KIDNEY DISEASE

A gratifying feature of successful kidney transplantation is restoration of sexual function and the opportunity of parenthood. In the past there have been few reports of parenthood in kidney transplant recipients. But Dr. Thomas E. Starzl of the University of Colorado has now described 33 pregnancies in families with 8 female and 19 male kidney transplant recipients. Of these, 25 have resulted in live births, 5 are still in the gestational state, and 3 have ended in abortions.

Eighteen of 19 infants fathered by male transplant recipients were normal. By contrast, of 6 infants born to female transplant recipients, only 2 had a completely uncomplicated first few weeks of life. Of the others, one died shortly after birth from hyaline membrane disease and one had a congenital anomaly. Kidney function underwent deterioration in 3 women during pregnancy, but these abnormalities receded following either delivery or abortion.

Based on this study, male transplant recipients are now advised that their prospects of having normal children are excellent. Both male and female recipients, however, are told that their own long-term prognosis and consequent prospects of raising their offspring are still not known. Female transplant recipients are told, in addition, that risks in general and particularly in the first few weeks of life are greater to their children and that there may be some significant hazard to the transplanted kidney during gestation.

## Dialysis Growth Failure

Serious growth failure and failure to attain pubertal maturity has been consistent in children receiving chronic hemodialysis (blood cleansing with artificial kidneys). Largely for this reason children under age 15 usually are not accepted for such treatment. Traditionally, growth failure has been attributed to hormonal deficiencies or imbalances resulting from dialysis.

Dr. Malcolm A. Holliday of the University of California, San Francisco, now had demonstrated that the growth failure can be obviated if the dialyzed children consume a full complement of calories. Five children with calorie intakes of less than 70% of recommended daily allowances grew at an average rate of only 43% of normal, while 8 children with calorie intakes of 70% or more of recommended daily allowances grew at an average rate of 113% of normal, during a 3-15 months period of observation.

Dr. Holliday concluded that there is an apparent borderline of calorie intake below which children on dialysis grow poorly, but that growth failure can be corrected through an adequate nutritional intake.

## Uremia and Dialysis

Several other findings involving chronic kidney failure and hemodialysis also are worthy of note. Drs. Ralph S. Goldsmith and Claude D. Arnaud of the Mayo Clinic, Rochester, Minnesota, have shown that the hypersecretion of parathyroid hormone associated with chronic kidney failure can be controlled by simultaneously decreasing blood phosphate through oral administration of aluminum hydroxide and increasing dialysate calcium levels. This approach might avoid the necessity for surgical removal of the parathyroid glands.

At the New York Medical College, Dr. Kurt Lange has shown that the blood and spinal fluid of patients with chronic uremia contain a dialyzable toxic factor that inhibits activity of the enzyme transketolase in nerve tissue. This finding may help to explain the neurological damage associated with chronic uremia, and may also provide a means of testing the efficiency of a particular hemodialysis regimen.

Dr. Neal S. Bricker of Washington University, St. Louis, has described a unique blood fraction obtained from patients with chronic uremia which inhibits sodium transport in the isolated frog skin. Such a substance, if found to be present in quantity, could be responsible for several symptoms and signs of the uremic state.

# ENDOCRINOLOGY

In attempting to understand any disease process or physiologic disorder it is necessary to consider the possible role played by the various hormones, as well as the factors which regulate their synthesis, release, and degradation. The processes of isolation,

purification, characterization, and synthesis of hormones are continuing at a rapid pace, and the few remaining unidentified endocrine factors are succumbing to the latest research advances.

## Prolactin

Prolactin is an anterior pituitary hormone which stimulates milk secretion in mammals. It has been known since 1961 that human pituitary growth hormone also stimulates milk secretion, but efforts to isolate a separate human prolactin from growth hormone have been fruitless. In fact, doubt has been expressed that humans possess a separate prolactin-like hormone comparable to that of lower animals.

Development of a highly sensitive bioassay for prolactin in human blood now has provided evidence that prolactin exists in man and that it circulates in the blood independently of growth hormone. These studies by Drs. Andrew G. Frantz and David L. Kleinberg of Columbia University also indicate that circulating growth hormone, like that extracted from human pituitary glands, also has the ability to stimulate milk secretion.

Meanwhile, Dr. Judson J. Van Wyk of the University of North Carolina has shown that surgical transection of the pituitary gland stalk in man (for suppression of breast cancer or diabetic retinopathy) results in as much as a 100 fold rise in blood levels of prolactin, with negligible changes in blood levels of growth hormone. Dr. Van Wyk's observations constitute further evidence for the existence of a human prolactin molecule as a separate hormonal entity distinct from growth hormone.

# NUTRITION

## Progress in Vitamin D Area

Vitamin D acts to maintain normal bone mineralization and metabolism by facilitating and regulating the absorption of calcium and phosphorus from the intestinal tract. About 2 years ago, the principal biologically active form of this vitamin, 25-hydroxycholecalciferol, or 25-HCC, was isolated from plasma by Dr. Hector F. DeLuca of the University of Wisconsin. He subsequently showed that vitamin D is converted to 25-HCC in the liver, and that the metabolite may be a safe and effective form of therapy for vitamin D-resistant rickets.

Almost simultaneously, Dr. DeLuca and two other NIAMD grantees now have obtained evidence to indicate that 25-HCC is converted in kidney tissue to a metabolically still more active form of vitamin D, namely 1,25-dihydroxycholecalciferol. The newly described metabolite may represent the ultimate biologically active form of vitamin D in the intestine. It is more than twice as active as 25-HCC in stimulating intestinal calcium transport.

This finding should afford several new avenues of experimentation It should now be possible to synthesize the new metabolite, to determine its precise mode of action and its relationship to other

known regulators of calcium metabolism, and to determine its efficacy in the treatment of several diseases resulting from abnormal vitamin D metabolism.

## Protein-rich Foods for Underdeveloped Countries

Through NIAMD support, the Central Food Technological Research Institute in Mysore, India, has developed a protein-rich "stretched" milk that could double the locally available milk supply for Indian children and those of other developing countries.

The new vegetable protein-toned milk, "Miltone," is regular buffalo milk (or in some cases cows' milk) which has been "stretched" through addition of an equivalent quantity of peanut protein isolate (pure peanut protein, a nutritionally desirable protein when mixed when some milk protein). This "toning" permits doubling the volume of the final product, to which vitamins, calcium, and hydrolized starch syrup are added as a source of extra carbohydrate.

This development won the 1971 Industrial Achievement Award of the Institute of Food Technology - the American and International professional organization.

## New High-Protein Rice

Commercial varieties of rice contain only from 7 to 7-1/2 percent protein. Because the biological quality of rice protein is fairly high, an increase in the protein content of rice by several percentage points would go far toward alleviating protein undernutrition in countries in which rice is the mainstay of subsistence. Such an accomplishment could result in an automatic, sweeping nutritional improvement in areas inhabited by 60 percent of the world's population.

A high-protein rice developed in an NIAMD - administered contract project of the U. S.-Japan Cooperative Medical Science Program now has been made available for feeding tests in adult human subjects. Results obtained in Purdue University students indicate that the high-protein rice, which contains 1.8 times as much protein as a conventional commercial variety, causes significantly higher nitrogen retention (tissue protein buildup).

Dr. Helen F. Clark of Purdue University, who reported this finding, utilized milled high-protein rice grown under NIAMD sponsorship by the International Rice Research Institute in the Philippines.

## Maternal Protein Deprivation

Many studies have indicated that maternal protein deprivation before and after birth results in mental impairment and brain cell deficiency in children.

After a study in rats, NINDS-supported scientists report that both first and second generation offspring of protein-restricted

mothers are born neurologically handicapped. If results in rats are relevant to humans, even though the offspring's nutrition is improved, a cerebral deficiency may last for at least one generation more.

In an earlier study, investigators at the Brain Research Institut and Mental Retardation Center in Los Angeles had shown that when female rats are maintained on a low protein diet one month prior to mating and throughout pregnancy, their offspring at birth had significantly lower body weights and cerebral weights, and less cerebral protein and cerebral deoxyribonucleic acid (DNA).

This research has now been extended to study the effect of maternal dietary protein restriction on cerebral DNA and cerebral protein in the second generation offspring.

The investigators believe that the cerebral impairment in the second generation animals was due to handicaps present in the first generation female rats at birth because of protein deprivation in their mothers. These handicaps may have been in the kidney or in systems necessary to utilize food efficiently. According to the investigators, the first generation rats suffered from "cryptic malnutrition," so that they maintained a deficit even when given full access to normal food after birth.

Supported by both NINDS and NICHD, the work was carried out by Drs. Stephen Zamenhof, Edith van Marthens, and Ludmila Grauel, of the Brain Research Institute and Mental Retardation Center, UCLA, Los Angeles.

## Guatemala Nutrition Study

In 1964 the National Institute of Child Health and Human Development, with cooperation of the Pan American Health Organization, began in Guatemala a study of the complex problems of malnutrition and its effects on physical and mental development. After extensive field work, the first definitive results on nutrition and pregnancy outcome have been obtained.

In Guatemala, 85 percent of all children under 6 are significantly underweight, 85 babies per 1,000 die within the first year of life, and life expectancy is only 37 years.

The investigations took place in 4 villages in the Guatemalan highlands, which had comparable populations, socio-economic and nutrition status. The villages were matched in pairs, with one in each pair receiving a liquid supplement to their regular diet. The supplement included protein and calories in addition to vitamins and minerals. The control villages also received a beverage (called fresco) which was fortified to provide a comparable level of vitamins and minerals but a lower level of additional carbohydrate calories. All villages received medical care, and the same amount of social stimulation from the investigating teams.

Data have been analyzed for 113 mothers who have delivered babies during the course of this project, 71 in the supplemented villages,

and 42 in the unsupplemented villages. It is apparent from the data that many factors influence the outcome of pregnancy, most importantly the sex of the child, maternal height, and number of previous pregnancies. These factors have been taken into account in analyzing the Guatemalan data. Malnutrition in these propulations is sufficiently pronounced that the birth weight of infants can be correlated with dietary intake during the last two trimesters of the mother's pregnancy. Maternal supplementation with the protein-calorie-vitamin-mineral mixture increased the number of high birth weight infants significantly. The birth weights of infants born to mothers who participated fully in the supplementation program were equal to birth weights in the U. S., approximately 300 grams more than found in the unsupplemented mothers. This is the first study which, by its design and analysis, has taken into account the important factors which influence pregnancy outcome; we can thus attribute the observed differences essentially to variations in nutritional status.

Studies have also been undertaken concerning the growth of young children. In this population all children are breast-fed as a primary source of food and weaning occurs at 15-24 months. After the age of 6 months there is a significantly greater rate of growth among supplemented children than in control children who participate equally well or better in the fresco program; this comparison minimizes effects such as relative social class, parental interest or illness. The increased rate of growth (height) by supplemented children is comparable to that in U.S. children and is about 10 percent greater than in the unsupplemented children.

All villages in this study were given a special medical program utilizing immunizations and preventive medical care, as well as treatment of specific illnesses. Such a health care program would be applicable not only to emerging nations where physicians are rare, but also to high density inner city populations in more advanced countries. In the Guatemala study, preliminary data suggest that this program decreased the infant and preschool mortality rates by more than half. It would appear that there is also a decrease in the frequency of preschool diarrhea in the supplemented villages. As yet the data are not adequate to permit assessment of the impact of supplementation on death rates, or on mental development.

## Sequel to the Dutch Famine

In a 1971 retrospective study of a war famine population in the Netherlands, two NICHD scientists recently sought to determine the relationships between mental performance, social environment, and nutrition. Data from the study emphasizes the powerful influence of social class and social environment on mental performance and IQ scores, but, surprisingly, they fail to reveal any nutritional influence.

In September of 1944, Dutch railworkers in Nazi-occupied territories conducted a rail strike in response to the call of their exiled government. The Nazi command retaliated by imposing an embargo on the movement of all goods, including foodstuffs.

No food could be imported into cities from their rural supply areas from November 1944 until April 1945, when liberating armies arrived.

The main impact of the famine was suffered by people in the large cities occupied by the Nazis, because rural areas still had access to food. The part of the country south of the Rhine River was occupied by the Allied armies and was not restricted by the embargo. Towns of the east and north were liberated earlier than those in the west, and had better access to food during the famine. The geographic distribution of the famine thus provided the investigators with a unique opportunity to study famine effects.

In famine cities of the Western Netherlands official rations were about 450 calories per day, only one-fourth of the minimum daily requirements. The death rate increased significantly during the famine.

Immediately after liberation in early May, clinical interview and examination of samples of the populations were conducted by mobile nutrition teams. Their carefully tabulated reports leave no doubt of the seriousness and severity of the physical and mental suffering.

Under a grant from the National Institute of Child Health and Human Development, Drs. Zena Stein and Melvyn Susser have now examined whether exposure to famine during gestation has a measurable effect on mental ability or intelligence quotient of children. The investigators followed the histories of 2,000 Dutch boys who were carried or conceived during the famine.

These offspring, some from famine cities and others from control cities, were examined at the time of military induction, at ages of 18 or 19, and tested for mental retardation and intelligence performance. The records indicate that those nutritionally deprived during gestation did not have any disadvantages with respect to mental retardation or IQ in comparison with those who were not exposed to the famine.

Conclusions from this study should not be generalized, the scientists observed. The report does not rule out the possibility that chronic nutritional deficiency in mothers could adversely affect the mental development of their offspring.

## NEUROLOGICAL DISEASES

Research into diseases of the nervous system produced important results during the year.

### PARKINSON'S DISEASE

Many elderly people who suffer the severe nervous disorder known as Parkinson's disease have been helped by the new drug levodopa or L-dopa. Prior to its widespread clinical use, there were indications that L-dopa tended to strengthen the heartbeat for

about two hours after administration, but the effect had not been studied in detail. While this effect was not considered harmful to most patients, there was some concern for its safe use in parkinsonism patients who might also have heart disease.

Accordingly, Dr. Leon Goldberg, a grantee of the National Institute of General Medical Sciences (NIGMS) at Emory University, has studied the problem of potential adverse cardiac effects from L-dopa. The results of his research are encouraging. On the one hand, it was found that the drug can lead to arrhythmia or irregular heartbeat, which could be fatal in persons with severe coronary disease. On the other, Dr. Goldberg discovered that the effect of L-dopa on the heart can be completely blocked by another drug, propranolol, without affecting its beneficial action on Parkinson's disease.

The experimental drug, MK-486, was found to increase the effectiveness of levodopa in treating parkinsonism patients so that the levodopa dosage can be reduced by 80 percent, grantees of the National Institute of Neurological Diseases and Stroke (NINDS) at Columbia University reported. One of the problems in using levodopa has been that the massive doses necessary to achieve control of parkinsonism symptoms have also caused side effects so severe that one-fourth to one-third of the patients have not been able to tolerate the drug.

MK-486 inhibits the normally rapid metabolic breakdown of levodopa in the body, thus affording more time for it to penetrate brain tissue, and making possible smaller doses. One of the most common side effects, irregular involuntary movements, remains unaffected, however, and scientists are now trying compounds similar to MK-486 in various combinations, in the hope of improving treatment, and of learning more about the disease process.

Dr. George Cotzias, developer of levodopa therapy for parkinsonism, and also an NINDS grantee, has found that the enzyme inhibitor, MK-486, permits coadministration of vitamin B6 with levodopa, and this has improved the mental state in some patients. One of the problems with levodopa therapy has been that vitamin B6 seemed to cancel out the levodopa effects, and had to be taken out of the diet. If remaining problems with MK-486 can be resolved, its use may be a significant advance in the therapy of Parkinson's disease.

## Muscle Tissue Research

Three distinct fiber types exist in human muscle, according to one group of NINDS grantees, rather than two types, as had been postulated by other scientists. The finding, by Dr. Michael Brooke and colleagues at the University of Colorado, is of importance in parkinsonism, because each of the three types has its own independent nerve supply, and is probably essential for motor control. The scientists are now attempting to find out whether there is selective atrophy or hypertrophy of the various fiber types in parkinsonism.

Using an improved device for measuring contraction time of muscles, they will attempt to determine the effect of levodopa on the abnormalities seen in the muscle tissue of 80 percent of parkinsonism patients.

## CEREBRAL PALSY

Expected birthweight may be a more important factor than gestational age for doctors to consider in deciding to induce labor early or resort to cesarean section, studies at The Johns Hopkins University Medical School and Hospital, in conjunction with the NINDS Collaborative Perinatal Research Project, suggest. Low birthweight and prematurity are known to be related to neurological deficits, including cerebral palsy.

Drs. Irvin M. Cushner and E. David Mellits have found that the risk of death among babies in their Collaborative Study population was greatest for those with low birthweight (1,500 grams--3.3 pounds--and under), regardless of duration of gestation. Furthermore, they found risks were acceptably low among infants in their study with higher birthweights (1,500 grams and over) regardless of pregnancy duration.

They conclude that intervention can be considered for a fetus in danger in its intra-uterine environment, provided it is at 33 weeks or more gestational age and can be assumed to weigh more than 1,500 grams. Their data suggest that under these circumstances delivery would be associated with an acceptably minimal risk of neonatal death or later neurological impairment.

### Animal Model

With the exception of athetosis, scientists have been able to produce most of the main motor disorders in monkeys, in order to study them. Athetosis is an involuntary and uncontrolled movement most often found in the heterogenous group of conditions termed "cerebral palsy."

An important finding was made recently by an NINDS grantee, Dr. Fred A. Mettler, Professor of Anatomy at Columbia University, who demonstrated that transitory athetoid movements can be produced in monkeys by making lesions in the basal ganglia and its pathways and by the administration of the drug sodium azide. His goal is to perfect a technique for producing a permanent atherosis.

### Levodopa Therapy

Levodopa, a therapeutic agent used successfully in Parkinson's disease, was recently used to treat 9 athetoid cerebral palsy patients at the Hospital for Special Surgery affiliated with the New York Hospital-Cornell Medical College, New York. It was thought that levodopa might help patients with athetoid cerebral palsy and might ameliorate some, if not all, of the manifestations of the disease--rigidity, dystonia, and tremor.

In a preliminary report (Neurology, January 1972) the investigators said 8 of the patients showed varying degrees of improvement, confirmed by double-blind placebo trials. Even though no patient totally lost his athetosis, the overall function of each patient improved; symptoms were significantly reduced. Common side effects, loss of appetite, nausea, and vomiting, were present in all patients and were related to drug dosage. The patients themselves believed the treatment with levodopa was beneficial. The improvement is being sustained on continued treatment. One of the investigators, Dr. Fletcher H. McDowell, is a grantee of the NINDS.

## AMYOTROPHIC LATERAL SCLEROSIS

Drs. Paul M. Hoffman and Jacob A. Brody in the NINDS Epidemiology Branch, Collaborative and Field Research, analyzed death certificates of a series of patients in whom amyotrophic lateral sclerosis (ALS) had been diagnosed during life, to determine the usefulness of mortality statistics in estimating the prevalence of ALS.

The investigators reviewed hospital records of deceased patients from North Carolina who had had definite diagnoses of ALS. This included certainty of progression, bulbar signs and symptoms, evidence of long tract involvements, and electromyographic or muscle biopsy evidence of widespread neurogenic atrophy. These were then correlated with information on the patients' death certificates.

They found only 72 percent of the death certificates listed ALS as a cause of death. The data further indicated that clinically diagnosed ALS may be mistakenly recorded as multiple sclerosis on death certificates. The researchers, noting that mortality data even in diseases with distinct clinical manifestations, such as ALS, must be interpreted cautiously, concluded the true death toll from ALS probably is considerably higher than now reported.

## NEUROMUSCULAR DISEASES

### Muscular Dystrophy

Progress in research to reduce the number of unidentified carriers of Duchenne muscular dystrophy--an inherited muscular disorder of children--and to simplify detection methods was reported in the year.

NIH grantees at the University of Iowa, Victor Ionasescu, Hans Zellweger, and Thomas W. Conway, found a carrier detection test which is potentially superior to methods now available, by examining the protein-making activity of the muscle polyribosomes in muscular dystrophy patients and suspected carriers. They found that polyribosomes from the patients showed increased protein-making ability. This, according to the scientists, may be an index of muscle fiber regeneration in a disorder characterized by muscle degeneration. The polyribosome activity in the suspected carriers was also increased.

When the protein synthesizing ability in potential carriers was compared with their serum enzyme determinations (the most widely used carrier-detection method) it was found that the polyribosome determination was a more sensitive test of the carrier state.

## Polymyositis

Polymyositis, a chronic, inflammatory muscle disease characterized by remissions and flare-ups, leads to muscle weakness and a progressive downhill course. NIH grantees at the University of California, Drs. Murray C. Sokoloff, Leonard S. Goldberg, and Carl M. Pearson, reported that methotrexate, an immunosuppressive drug with anti-inflammatory effects, is an effective treatment. Seven patients with polymyositis who had failed to respond to corticosteroid therapy --the usual treatment--were given intermittent intravenous methotrexate. Five improved significantly in muscle strength, return of serum enzyme levels to normal or near normal, and ability to reduce gradually the amount of corticosteroid without deterioration. Toxic side effects were well controlled. Because 4 of the 5 were on a corticosteroid when methotrexate was started, it is possible, according to the investigators, that corticosteroid therapy is required for methotrexate to exert its beneficial effects.

## Myasthenia Gravis

Some scientists believe that myasthenia gravis--a neuromuscular disease characterized by weakness and abnormal fatigue of the voluntary muscles--is an autoimmune disease, that is, that the body itself may be producing a protein or other substance which interferes with proper muscle activity. An animal model recently developed by NINDS grantees at New York University School of Medicine, Drs. Gideon Goldstein and Andres Manganaro, may aid studies of the immunological aspects.

These investigators found that a protein, "thymin," extracted from the thymus (a gland believed to play a role in the body's defense system), when injected into pigs and rats resulted in a condition in the muscle-nerve junction closely resembling human myasthenia gravis. This suggests that myasthenia gravis may be a disorder in which the body causes inflammation of its own thymus gland and overproduction of a proposed thymic "hormone." This hormone in turn may interfere with transmission of nerve messages to muscle, causing muscle weakness.

Historically myasthenia gravis has not been considered an inherited disorder. Studies by an NINDS grantee at the University of California at Los Angeles, Dr. Christian Herrmann, Jr., indicate, however, that it occurs in families more commonly than can be explained by probability.

Reviewing cases reported in medical literature and histories of 353 patients at UCLA over a 16-year period, the scientist found the disease occurred in siblings, in both parent and child, among more distant relatives such as cousins, and in identical twins. Myasthenia gravis was generally found in only one of male fraternal

or identical twins, but both female identical twins were affected. This data is insufficient to confirm the usual genetic mechanisms, but does suggest that an environmental factor or factors may work in combination with a hereditary predisposition to cause the disease.

## Treatment of Myasthenia Gravis

Myasthenia gravis (MG) is the most successfully treated of all the neuromuscular disorders. A number of drugs are effective for many patients, and therapy for others is under study at several centers. NINDS grantees at Massachusetts General Hospital and Mt. Sinai Hospital, Drs. Vincent P. Perlo, Barry Arnason, David Poskanzer, Benjamin Castleman, Robert S. Schwab, Kermit E. Osserman, Angelo Papatestis, Lawrence Alpert and Alan Kark, reported on the role of thymus removal by surgery.

The thymus gland is an organ located near the heart which normally atrophies with maturity and is believed to be involved with the body's defense system. Its removal is beneficial to many patients In the new report, the surgery was beneficial in 89 percent of 267 patients, most of them with a severe degree of the disease. This study, covering a four-year period, showed that improvement, once attained, remained permanent in all but one case.

Of the patients, 239 had the removal through a split in the chestbone--the standard method--and 28 through an incision in the neck, a recent development.

Results with this new technique compared favorably with the standard method and the surgery was less traumatic. The surgery succeeded in patients who had had symptoms five years or more and results were comparable in men and in women. Candidates for surgery were 10 to 60 years old.

Other NINDS grantees reported that the germine acetates (which belong to a group of drugs called veratrum alkaloids) can cause improvement in neuromuscular function in patients with MG. These have an advantage over other veratrum alkaloids in that they do not cause slowing of heartbeat or increased blood pressure. Patients treated in the study reported improvement in carrying out the activities of daily living such as climbing stairs, eating, and tooth-brushing.

ACTH (adrenocorticotropic hormone) is a widely used therapy for myasthenia gravis patients. Following experience with more than 100 patients given some 300 courses of treatment over the past 6 years, a group of NINDS grantees reported that ACTH improved muscle strength, beginning 10 days after treatment was started.

A consistent finding, however, was that about three-fourths of the patients initially experienced respiratory distress and a marked decrease in muscle strength. Paradoxically, the patients with the greatest initial weakness later experienced the most striking benefit. ACTH causes the adrenal glands to release steroid hormones which appear to bring about improvement in myasthenia gravis patients.

Treatment with a steroid, prednisone, has been shown by a NINDS scientist, Dr. W. King Engel, to be highly effective when given on a schedule which minimizes side effects. Ten patients, formerly bedridden, have been able to lead normal lives for several years since they started prednisone therapy.

## INBORN ERRORS OF METABOLISM

### Gaucher's, Niemann-Pick, and Fabry's Diseases

Three genetic abnormalities causing severe neurological disorders have been diagnosed in unborn babies by NINDS scientists under the direction of Dr. Roscoe O. Brady, Assistant Chief of the Laboratory of Neurochemistry. These are Gaucher's, Niemann-Pick, and Fabry's diseases.

Research developed over a period of more than 4 years has made it possible to identify the metabolic defects responsible for the accumulation of lipid materials in these disorders and in Tay-Sachs disease. Over 50 pregnancies monitored because of family history revealed 4 cases of Niemann-Pick disease, 2 of Gaucher's, and 1 of Fabry's disease in the second trimester (3 to 6 months) of pregnancy. A half-dozen pregnancies were monitored for Tay-Sachs disease, but none of the fetuses were found to be affected.

Pregnancy monitorings are performed by obtaining with a needle and syringe a small quantity of cells from the fluid surrounding the fetus--a process known as amniocentesis. The cells are grown in tissue culture for several weeks until enough are available for accurate chemical analysis. They are then harvested, and an enzyme assay is performed to detect the particular deficit in each disease.

The 7 pregnancies in which the diseases were identified were terminated. Accuracy of the prenatal diagnosis was confirmed in all cases.

### Metachromatic Leukodystrophy

Metrachromatic leukodystrophy (MLD) is a hereditary lipid-storage disease due to the lack of a specific enzyme which normally breaks down certain fatty material. It leads to severe mental retardation and early death.

NINDS grantees at Pacific State Hospital, Drs. Myna T. Porter, Arvan L. Fluharty, and Hyato Kihara, have developed a skin cell culture which indicates that enzyme replacement therapy may be feasible for this and related lipid-storage diseases.

In MLD, substances called cerebroside sulfates accumulate in the peripheral and central nervous system, due to the lack of an enzyme which normally breaks down the sulfatides. This enzyme deficiency has been found by the scientists to exist also in skin cells taken from MLD patients.

In culture of skin cells, the investigators were able to correct

the biochemical defect by adding the necessary enzyme. After special handling, the MLD cells were then able to utilize the added sulfatides just as normal cells or cells with only partial enzyme deficiency. These studies show for the first time that the enzyme can penetrate an intact skin cell grown in tissue culture.

## MULTIPLE SCLEROSIS

Investigations conducted by NINDS researchers into the metabolism of proteins found in the myelin sheath (the covering around certain nerve cells) have yielded results that modify time-held theories on how the myelin sheath is formed.

Studying the metabolism of myelin basic protein from various regions of the central nervous system of both immature and adult rats, Dr. Roscoe O. Brady, Assistant Chief of the Laboratory of Neurochemistry, NINDS, has found that the protein is made and broken down very rapidly. He and his co-workers, R. Sammeck, a visiting scientist from Germany, and Dr. R. E. Martenson, Section on Myelin Chemistry, Laboratory of Cerebral Metabolism, National Institute of Mental Health, conclude that the general impression of stability of the myelin sheath components must be drastically revised, and that there may be precursors of the myelin basic protein.

During the course of the research Dr. Brady and Dr. Richard H. Quarles, also with the Laboratory of Neurochemistry, disposed of still another misconception regarding the composition of the myelin sheath. It had been generally accepted that lipids and proteins are present. This team discovered that sugar-linked proteins are definitely contained in the myelin sheath, and of these, some may be present in particularly high concentration.

## EPILEPSY

Epilepsy is a term used to describe both a set of symptoms (seizures) and the underlying mechanism that causes them: recurring, abnormal, uncontrolled electrical discharges of brain cells.

Further proof that brain glial cells probably play an important role in epilepsy mechanisms was afforded by research done this year by Drs. Emil C. Zuckermann and Gilbert H. Glaser at the Yale University Medical School under an NINDS grant.

From previous experiments, the researchers knew that relatively small increases in potassium concentration in cerebrospinal fluid (CSF) in cat brains can cause seizures, and that normal brains probably have functional barriers protecting nerve cells (neurons) against potassium accumulation.

In this experiment, the investigators first induced epilepsy in 40 cats chemically. Then they compared the reactions of those cats with the reactions of 60 normal control animals when solutions of varying concentrations of potassium were injected into their brains. The concentration needed to trigger seizures in some animals in both groups was about the same. However, with each concentration above

that level, the percentage of individual cats reacting with seizures was double in the epileptic cats. In the epileptic cats there was also a significant decrease in the time between injection and seizure onset and a much greater number of repeat seizures after potassium injection was stopped.

Conclusions were that the scars on the brains of the epileptic animals disturbed the functions of the glial cells which normally prevent a large increase of potassium in the extracellular areas of the brain.

## Diagnosis of Epilepsy

Drugs have long been used to treat epilepsy. Now, a drug has been used successfully to diagnose the disorder.

Brevital (methohexital), a fast acting barbiturate, normally used as an anesthetic, will activate generalized epilepsy-seizure electrical discharges in most persons who have generalized (petit mal and grand mal) epilepsy. The discovery was made by Institute grantees, Drs. B. J. Wilder, L. Musella, Gage Van Horn, and R. P. Schmidt, of the Veterans Administration Hospital and the University of Florida School of Medicine, Gainesville, Fla., and the University of Pittsburgh.

When the investigators gave small doses of the drug to 39 persons who had histories of generalized epilepsy and to 40 normal controls while EEG's were being recorded, 34 of the epilepsy group generated characteristic discharge patterns, while the patterns of the control group were not affected. Further, comparison of these results with similar earlier tests with persons who had histories of partial (psychomotor) epilepsy showed that much smaller doses were needed to activate the abnormal discharges in cases of generalized epilepsies.

The technique is considered safe and effective and its developers expect it to become a valuable tool in making quicker, more positive conclusions.

A method to compress a 140-page record of brain waves or electroencephalograms (EEG's) into a computerized, three-dimensional, topographic presentation on a single page was developed this year by Dr. Reginald G. Bickford, Director of Research of the Department of Neurosciences at the University of California at San Diego Medical School, and his staff with a grant from NINDS. This procedure also provides computer analysis of over 50 percent of EEG's in seconds, a process which would normally require much longer even if done by experienced human encephalographers.

The Compressed Spectral Array (CSA), as the plot is called, is an application of two computer uses developed by others--frequency spectral analysis and hidden-line suppression. The latter was first applied to EEG data by a Stanford University pharmacologist two years ago.

Dr. Bickford claims that the new method has these advantages: (1) a simplified pictorial display of the clinical EEG on a single page, easily understood by a clinician who has not had extensive EEG training, (2) a quantitated EEG providing data which can later improve EEG diagnostic accuracy, (3) a machine which operates well in the presence of muscle and machine effects and (4) dynamic EEG features which are retained as the EEG changes during recording.

## Prevention of Epilepsy

There is increasing interest in studies to learn whether drug treatment can prevent epilepsy which often follows serious head injuries.

Among investigators pursuing possibilities of prevention were Dr. J. Kiffin Penry, Chief, Applied Neurologic Research Branch of the Institute's Collaborative and Field Research, and his staff associate, Dr. R. L. Rapport II. In a paper, "Pharmacologic Prophylaxis of Posttraumatic Epilepsy," prepared for publication in Epilepsia, they reviewed the history of posttraumatic management. The review disclosed that only 3 controlled investigations of head-injured patients have been made since 1947 in which the efficiency of drug treatment of posttraumatic epilepsy has been evaluated. The best results in preventing first epileptic convulsions were in a program where both phenobarbital and diphenylhydantoin were used.

The authors concluded that no adequate statistical evaluation of the problem can be based on the three cited studies because of limitations in test design or population size. They argue that the large number of Americans (over 101,000 in 1970 alone) who have suffered head injuries which have resulted in or will result in at least one convulsion demands attention to the problem.

## Treatment of Epilepsy

A new diet for managing difficult epilepsy cases has been developed to replace the so-called ketogenic diet used since 1921. This high-fat diet was designed to put into a patient's system excesses of chemical elements called ketones which have some effect in seizure control. The diet is still one of the most effective therapies for hard-to-manage seizures in children. However, it restricts carbohydrates so much that it tastes bad and is hard to prepare. Very few children can accept it for long periods.

Last year NINDS grantees P. R. Huttenlocher, A. J. Wilbourn, and J. M. Signore, of the Department of Pediatrics and the Section of Neurology at Yale University School of Medicine devised a new diet which uses tasteless chemicals called medium-chain-triglycerides that induce ketones into the system more readily than do dietary fats. Because the new diet allows substantial reduction of dietary fats, carbohydrates can be greatly increased, making the foods more palatable and easier to prepare.

In tests completed on 12 children and adolescents between 2 and 16 years of age who had failed to respond to all conventional

therapeutic methods, antiseizure effects of the new diet have proved as good as, if not superior to, the old one. It has been especially effective in two types of generalized seizures-myoclonic or akinetic--with minimal side effects.

## COMMUNICATIVE DISORDERS

### Sensori-Neural Hearing Loss

A person with sensori-neural hearing loss has difficulty understanding words but often can hear loud sounds. This type of loss results from damage to the inner ear, or the nerves leading from the ear to the brain, or the brain itself.

Noise has been shown to be an important cause of sensori-neural hearing loss. Such loss can be either temporary or permanent depending on the level of noise and the length of exposure. Temporary loss is often referred to as "auditory fatigue." Noise causes hearing loss by damaging hair cells in the inner ear.

Extensive studies by Dr. Dixon Ward, University of Minnesota, an NINDS grantee, indicate that steady noises above 80 decibels are capable of producing temporary hearing loss. Steady noises above 105 decibels produce permanent damage in a normal, unprotected ear exposed 8 hours a day for several years. Some recovery can occur after 2 weeks of absence of noise, but after that little additional recovery occurs even after a month of noise-free life; 16 noise-free hours are required for recovery from temporary hearing loss. Thus in an industrial situation some workers may have a chronic auditory fatigue that could lead to permanent damage.

Dr. Ward also found that: Women who have worked for years in a given noise show less permanent losses than men experiencing the same exposure. A single explosion can produce sensory hearing loss indistinguishable from loss developed after years of exposure to steady noise. A severe head blow can produce the same type of loss as an explosion. By age 50, 19 percent of all males exposed to routine everyday noises have a hearing loss which exceeds that at which industrial compensation begins.

Another cause of sensori-neural hearing loss may be inadequate blood flow in the inner ear. A new technique has been developed by Dr. M. Lawrence of the Kresge Hearing Research Institute and the Department of Otolaryngology, University of Michigan. Removal of a small wedge of bone from an inner ear structure known as the round window permits observation by a microscope and photography with a mounted camera. The technique is expected to provide important information on the relationship between blocked blood vessels and various drugs, oxygen distribution, and other factors affecting hearing.

### Environmental Noise Pollution

It has only recently been recognized that noise is as much a factor of environmental pollution as are noxious gases,

contaminated water, and soil. At present very little is known of the specific or general effect of noise on man's health and well-being. Studies on hearing loss represent only a minute portion of the spectrum of effects of noise, including physiological and psychological effects. Additional information is needed to define noise limits in order to safeguard health. These limits must cover not only excessive noise but also continued or repeated exposure to stress-producing noise. It has been demonstrated that noise can trigger cardiovascular and neurological changes. Very recent research has established that the developing human fetus is subject to many of the same auditory stresses that affect adults, with long-term influences on learning and other behavioral characteristics. At issue is whether repeated noise-induced changes of this nature ultimately may have cumulative effects.

The causes of sensorineural hearing loss in young children are frequently obscure. Research undertaken by Dr. Stephen A. Falk of the National Institute of Environmental Health Sciences (NIEHS) in collaboration with Dr. Joseph C. Farmer, Jr., of Duke University Medical Center is to focus attention upon noise from infant incubators as a possible factor, particularly in premature infants.

Analysis of noise from functioning incubators revealed an average noise level that does not exceed the minimal sound intensity thought capable of producing sensorineural hearing loss in adults regardless of duration. (Closing incubator doors and crying babies produced higher sound levels). The adult criteria, however, are not applicable to noise exposure in infants. As far as known, there have been no controlled studies concerning the effects of noise on the young or newborn animals. Continuous noise is more damaging than intermittent; damage-risk criteria established in adults are based on 8-hour per day exposures. A premature infant who is confined to an incubator for continuous periods of weeks or months may thus be put to an additional risk.

Some researchers have recently demonstrated a large potentiation of hair cell damage in guinea pigs upon exposure to noise and the antibiotic kanamycin, compared to exposure to either agent alone. The use of antibiotics that are potentially damaging to the ear in the newborn who is constantly exposed to noise in an incubator is a very analogous situation. Work is continuing on this project in an attempt to learn what effects, if any, noise from an incubator has on infant hearing and to establish damage-risk criteria for infants.

Another NIEHS researcher, Reginald O. Cook, is developing devices for measuring impedance of the middle ear. Present devices are limited to very low frequencies, are unwieldy, require very precise measurements of ear canal volume and near immobilization of the subject, and are limited as to range of atmospheric pressures. Work is progressing on a prototype of a device capable of generating, sensing, and transmitting the necessary pressures and velocities.

## Loss of Hearing in Children

Children form their language base between their second and fifth years of life. They are never again as ready, flexible, or adaptable in learning language. Learning to talk involves hearing words, recognizing them, assigning them meaning, and then using them to communicate. Therefore, it is imperative that any problem relating to the communication process be discovered early.

A team of researchers at Harvard University Medical School has compiled extensive data indicating that early speech screening combined with future testing helps identify children with poor speech and poor language comprehension who may also have underlying neurological and psychological problems. Drs. Miriam F. Fiedler, Eric Lenneberg, Ursula Rolfe, and James E. Drorbaugh developed a unique screening program as part of the NINDS Collaborative Perinatal Research Program. Information was gathered on the infant's and mother's medical history. Trained interviewers then tested 475 children in their homes at age 3, and again at age 8. Nine percent of the children tested at age 3 evidenced delayed speech. These children more often had mothers who had experienced an abnormal pregnancy, or difficult labor, or came from abnormal home conditions than those who had normal speech. They also had a higher incidence of neurological and psychological differences, as evidenced on retesting, indicating that early testing is reliable indicator of some of the underlying causes of speech problems.

## Aphasia

Aphasia, a language disorder usually resulting from injury or damage to the left side of the brain, affects a person's ability to assign meaning to words, organize words into thoughts, or repeat words. A current study by Dr. Marcel Kinsbourne at Duke University, a grantee of NINDS, suggests that the type of aphasia depends on the ability of the right side of the brain to take over language function from the damaged left side. He states that both sides of the brain may initially be capable of assuming language function, but that the left side usually successfully dominates. The ability of the right side to compensate varies when the left is damaged, and this variance may express itself in different types of language problems.

## Central Nervous System Damage

The nature of hearing loss resulting from damage to the brain is being studied in 100 patients with multiple sclerosis (MS) by NINDS grantees at Northwestern University. Their results show that hearing loss which occurs during episodes of the disease ends during periods of remission, when MS symptoms disappear. They believe that the area of the brain responsible for impaired hearing returns to normal during this time. The hearing loss may initially result from swelling which impairs the function of these nerves rather than from actual degeneration of the nerves in the brain, or whatever other mechanism accounts for all transient and remitting symptoms in MS.

The study is also providing information on how persons with this

type of hearing loss perform on hearing tests. With this information scientists hope to develop new tests to measure accurately the extent of central nervous system involvement.

## New Test for Deafness

A useful by-product for speech research and other branches of medicine has come from a program designed to study the heredity of human craniofacial problems. This new method that detects deafness in living animals was developed by Dr. Kenneth Brown of the National Institute of Dental Research and Eye Research Foundation scientists supported by the NINDS. Because the test, which enables geneticists to determine patterns of hereditary deafness in animals, does not require patient cooperation, it should prove useful in studying hearing in human infants as well.

### MEMORY

Studies conducted by NINDS grantees have given further credence to the theory that memory may be related to a part of the brain known as the dominant (left) ventrolateral thalamus.

Drs. George A. Ojemann, Katharine I. Blick, and Arthur A. Ward, Jr., at the University of Washington in Seattle, maintain that a link exists between two functions involved in short-term recall, and that this link may control what is received and transmitted. These two functions are "tagging" the material as important at the time it is presented, and "searching" (sorting through remembered information for the correct response).

Their conclusion is based on electrical stimulation studies. Stimulation during verbal presentation directs attention to the material being presented. This has the effect of labelling the material as unusually important--something worth remembering--and therefore makes material easier to select at the time of recall. Stimulation during recall, however, makes selection of the correct word more difficult because it speeds up the search process to the point where it becomes defective.

The authors conclude that the link between the functions of search and tagging, both of which occur in the left ventrolateral thalamus, may actually control short-term verbal memory.

## ALLERGY AND INFECTIOUS DISEASES

Important progress was made against several diseases caused by infectious organisms and by allergic responses and in related basic research.

### NEW VIRUSES

One of the most notable research successes of the National Institute of Allergy and Infectious Diseases (NIAID) occurred last year in the Institute's investigation of epidemic non-bacterial

gastroenteritis. An organism causing this disease was discovered by Dr. Neil Blacklow and others and named the Norwalk agent, since it was found in bacteria-free material from patients in an outbreak in Norwalk, Ohio. Although the organism has not yet been grown in tissue culture, studies have shown that the Norwalk agent has properties of a small virus which confers short-term immunity only. Specimens from other outbreaks of non-bacterial gastroenteritis--a major public health problem -- will be studied and the relationship of any new organism to the Norwalk agent will be investigated.

NIH-supported (NIAID and NCI) scientists in Madison, Wisconsin have reported isolation of what appears to be a new virus from the brain of a patient who died as the result of progressive multifocal leucoencephalopathy (PML) and Hodgkin's disease. Although the cause of PML -- a rare but fatal neurological disease -- had not been proven, electron microscopic studies of brain cells have led to strong suspicion that the disease is a viral infection. The new virus, isolated by Dr. Duard Walker and others and grown in tissue culture, resembles viruses of the papova group. These include polyoma and SV-40, both of which cause cancers in laboratory animals. More extensive studies of the virus' characteristics are needed to establish it as a new papova-virus and to determine its role, if any, in causing PML.

## BACTERIAL DISEASES

In spite of the proliferation of multi-purpose antibiotics and increased attention to sterile techniques, infections continue to be a major cause of disease and death in newborn babies. Infection rates in infants are now one-to-five per 1,000 live births, and fatality rates are reported to be about 40 percent of all cases, and as high as 60 percent in cases complicated by meningitis.

It is understandable, therefore, that when seven serious cases of a bacterial infection appeared in a single well-baby nursery over a nine month period, NIAID grantees were prompted to reopen an epidemiologic investigation begun during a similar epidemic several years ago. Physical examinations and cultures were made of all nursery personnel, surface areas in the nursery were cultured, and air samples were taken regularly.

Only cultures from specimens taken from one staff member -- an admitting nurse -- contained Proteus mirabilis (the offending organism). The majority of infants who became ill had been bathed by this nurse at the time of admission to the nursery. Dr. John P. Burke and his associates who conducted the investigation at Boston City Hospital believe that transmission of the organism occurred during bathing of the umbilical stump. After the suspected carrier was removed from the nursery, there was no further problem with epidemic proteus infection.

Far removed from a well-baby nursery are other NIH-directed studies carried out by scientists in East Pakistan (now Bangladesh) before the recent conflict. Basing their studies of 16 cholera patients on earlier investigations in laboratory animals, Dr. L. C.

Chen and his co-workers at the Pakistan-SEATO Cholera Research Laboratory, Dacca, have found that an enzyme -- adenyl cyclase -- is a great deal more active in intestinal tissue specimens taken during the acute phase of cholera (when fluids are rapidly being lost) than in those obtained during the recovery period. The cholera toxin, or poison, seems to stimulate activity of the enzyme. This, in turn, affects the cyclic AMP system which controls a number of body secretions. The investigators suggest that, if their findings are confirmed, new treatments could be devised to inhibit activity of adenyl cyclase and thus block development of the life-threatening diarrhea of cholera.

At NIAID's Rocky Mountain Laboratory, scientists are continuing their inquiry into the immunology of tuberculosis. Drs. Edgar Ribi and R. L. Anacker and others, reported on use of living BCG tuberculosis vaccine given intravenously in monkeys to cause significant immunity. In comparative assays, they found that the tuberculosis cell wall vaccine, prepared at RML, compared favorably with live BCG vaccine. The scientists are now engaged in fractionating the tubercle bacillus in order to isolate and purify those components responsible for producing immunity.

The development in 1970 of the hamster as an experimental animal model for impetigo, a highly contagious bacterial skin infection, has made it possible to evaluate the many impetigo treatment methods in widespread use. In a study last year supported by NIAID and other Federal agencies, scientists found that benzathine penicillin, given by injections, best promoted the healing of impetigo in hamsters. Antibiotic salves were much less effective while other treatment, such as removal of impetigo scabs, was worthless. While Dr. A. S. Dajani and his co-workers at the University of Minnesota School of Medicine feel that use of penicillin to treat severe cases of impetigo is unquestionably warranted, they caution that the hazards of penicillin therapy -- such as an allergic reaction -- should be carefully weighed in management of patients with minor infections.

## FUNGAL INFECTIONS

Although fungal infections are widespread, they are most serious in patients whose immune response has been compromised by serious underlying disease and/or treatment with immunosuppressive drugs. Dr. Charles H. Kirkpatrick, Robert H. Rich, and John E. Bennett in NIAID's Laboratory of Clinical Investigations have been particularly interested in chronic mucocutaneous candidiasis, which usually occurs in such patients or in individuals with congenital defects that depress cellular immunity, an important part of the immune response.

Recently, in cooperative studies involving investigators at NIAID and other institutions, two types of defects in lymphocyte (white cell) function have been identified in individuals with chronic mucocutaneous candidiasis, and methods of immunologic reconstitution are underway. Massive transfusions of immuno-competent lymphoid cells have been given to some patients, with

encouraging results. However, this method is feasible only when a genetically matched donor -- a brother or sister -- is available. Other candidiasis patients have been given "transfer factor" -- a little-understood substance obtained from immune lymphocytes. Early results of transfer factor treatment are particularly encouraging since there is good evidence of restoration of cellular immunity.

Other fungi, ordinarily harmless, have been found to be pathogenic in immunologically compromised patients. Such an organism is Torulopsis glabrata. Identification of this common fungi as a threat under special circumstances was made by an NIAID grantee. Dr. Theodore C. Eickhoff and others at the University of Colorado Medical Center, over a 15-month period, studied isolates of T. glabrata from 37 patients and assigned the organism a pathogenic role in 11.

When cryptococcosis, popularly called "pigeon breeders disease," spreads to the tissue surrounding the brain, it is known as cryptococcal meningitis. Some chronic cases of this infection defy diagnosis by methods now in use. An NIAID awardee, Dr. M. G. Koenig, and investigators from the Center for Disease Control and the Veterans Administration reported last year that they have modified and evaluated a new latex agglutination detection test for cryptococcal antibody. The new test correctly diagnosed cryptococcal meningitis in patients whose cultures had repeatedly been negative for cryptococcal antigen; when antifungal chemotherapy was administered, recovery was complete. The investigators do not recommend abandoning the old diagnostic techniques but suggest that the new test be used as a concomitant check for infection, particularly since it is not known how often the others give false negative results.

## ALLERGY AND IMMUNOLOGY

Although immunology is not a new scientific discipline, the exciting role it plays in solving some of the medical mysteries of today is just beginning to be understood. As immunological techniques have grown and become more sophisticated they are being applied to the study of a variety of diseases.

### Allergy Studies

Historically, allergies were the first non-infectious diseases in which it was recognized that a better understanding of the immunologic system could lead to improved methods of treatment. Last year NIAID grantees at the University of Wisconsin reported some increased understanding of how ragweed pollen, in some individuals, causes not only hay fever but the more serious complications of bronchial asthma. Since ragweed pollen grains are too large to penetrate through the nose and upper airway passages to the bronchi, many scientists have postulated that irritation in the nose triggers an allergic constriction in the bronchi.

Drs. C. E. Reed and J. H. Hoehne found, however, in studies of patients deliberately exposed to increasing amounts of ragweed

pollen, that only when pollen grains were inhaled through the mouth did the coughing and breathing impairment characteristic of asthma occur. They found that the reaction is local and not an exaggerated reflex to nasal irritation.

This understanding of the means by which ragweed pollen causes a narrowing of the small bronchial tubes might well lead to the development of better means of preventing or treating this form of asthma.

Since it is impossible to analyze and dissect the allergic response in the whole man, scientists are increasingly directing their efforts to developing test tube techniques. Dr. L. M. Lichtenstein at Johns Hopkins University, Baltimore, has utilized white blood cells, more specifically the basophils which are a model for the target cell of importance in allergy -- the mast cell. To these white cells the scientists add antigens to which some individuals are allergic. The degree of sensitivity of a particular individual is indicated by the amount of histamine released by his cells; this chemical is known to be involved in allergic reactions and therefore constitutes an indicator of the allergic response. Relatively few laboratories are now equipped to do this kind of work. Dr. Lichtenstein and others, however, have shown a direct relationship between the sensitivity of a person's cells to a specific antigen and his allergic response following environmental exposure.

## Transplantation Immunology

Basic knowledge in immunology is contributing in an important way to the success of organ transplants. Ordinarily the body's immune system will reject tissue which it "recognizes" as "foreign" in much the same way that it protects the individual from invading disease-causing agents. "Matching" the donor and prospective recipient of a transplant is an important step in the process of tricking the immune system into accepting what it is geared to destroy. This matching is done by determining which of 4 major genetically determined transplantation antigens (HL-A) a donor and a recipient have in common, since many studies have shown that kidney transplants between HL-A identical siblings have a significantly improved survival as compared to the grafts from siblings which differ from the recipient in respect to one or two antigens.

During the past year a Dutch scientist, who is a NIAID contractor and a pioneer in the field, has suggested that grouping patients according to their immune potential and the frequency with which their HL-A profile occurs in the general population will not only improve treatment but will make results easier to interpret. Such a process would be especially valuable for cooperative regional programs in which data on prospective recipients and on donated organs are exchanged in order to achieve the best match. Dr. J. J. van Rood of the University Medical Center, Leiden, in attempting to explain why some grafts, mismatched for 3 or 4 antigens, are rejected and others, just as poorly matched, survive for many years, has proposed that the ability to respond strongly or weakly in the graft reaction may be determined by a genetic locus separate from that controlling the HL-A

antigens. In addition, previous blood transfusions may immunize the recipient against transplantation antigens present in a kidney he will later receive. (Conversely, under certain circumstances, these antigens may assist in the formation of circulating antibodies which can enhance the graft.)

However, if these factors, in addition to HL-A matching, are taken into account, Dr. van Rood suggests that patients can be divided into 4 categories: (1) those with a high immunological potential and a common antigenic profile; (2) those whose immune potential is low but antigenic profile is common; (3) those with high immune potential but rare antigenic profile; and (4) those with low immune potential and a rare antigenic profile. Patients in each of these categories would receive treatment designed to capitalize on their strengths and weaknesses.

Other NIH-supported investigators reported during the year that, in animals, long-term survival following bone marrow transplant is significantly diminished where recipients have previously received blood transfusions from well-matched donors. Since patients who are candidates for marrow transplants generally have received many transfusions as part of the management of their disease, Dr. E. Donnall Thomas and his associates at the USPHS Hospital, Seattle, believe that this finding may account for at least some of the instances where fatal graft-versus-host disease has occurred -- despite careful matching.

However, plasma transfusions have been successfully utilized by NIAID grantees to circumvent graft-versus-host disease an immunologically deficient infant was given incompatible marrow donated by her mother, since there were no HL-A identical siblings. Pre-transplant laboratory tests had shown that plasma from the donor prevented donor and recipient cells from reacting in the test tube; this suggested, and it was subsequently shown, that the maternal plasma contained antibodies that prevented the maternal cells from attacking the infant's tissues. Accordingly the patient was transfused with maternal plasma both before and after each of two transplants. The child improved dramatically and evidence of engraftment was obtained in the form of markedly improved immunologic capacity. The infant's immune deficiency was not wholly corrected, however and 14 months after transplant, she died of pneumonia. This infant's survival -- the longest on record for patients with severe immunodeficiency disease given incompatible bone marrow -- strongly supports usefulness of this approach. The work was reported by Drs. R. H. Buckley and her co-workers at Duke University School of Medicine, Durham.

In immunology research, soluble HL-A antigens are needed by scientists seeking to induce specific tolerance in a recipient of a donated organ.

Unfortunately, current production methods are extremely costly and do not yield sufficient soluble antigen fragments.

NIAID contractors have now reported preliminary data indicating

that fetal kidney may be a good source of soluble antigens. Basing their work on evidence that HL-A antigens are bound to embryonic cells in a fashion different from that in which they are bound to mature cells, Drs. M. A. Pellegrino, A. Pellegrino, and B. D. Kahan, at Massachusetts General Hospital, Boston, used low intensity sound to extract from fetal tissues water-soluble materials possessing HL-A antigenic determinants. Kidney preparations yielded the most potent antigens, with the fetal cells - unlike those in adult tissue - giving up almost 100% of their antigenic activity.

## PARASITOLOGY

An exciting preliminary finding by NIAID scientists during the past year has been the discovery of an agent, or agents, similar to bacterial viruses in pure cultures of the single-cell parasite, Entamoeba histolytica. The existence of this "virus" in several strains of the microorganism will have to be taken into account in future research on amebiasis -- the disease caused by E. histolytica. In addition, this discovery by Drs. Louis S. Diamond and his associates -- a first in protozoology -- provides virologists with a new system for investigating a naturally occurring virus in a relatively simple animal cell.

### Malaria

NIAID scientists also reported this year experimental transmission of African chimpanzee malaria to man by the bite of infected mosquitoes. Experimental infections with several nonhuman primate malaria strains have previously been reported, but they were all with parasites of Asian and New World monkeys.

This first successful experimental infection with parasites endemic to the African continent raises the possibility of its being zoonotic in Africa. Dr. Peter G. Contacos and his associates who carried out this experiment with the help of volunteers note with interest that neither of two African volunteers was susceptible to infection. These two cases were the only unsuccessful attempts to establish an experimental infection.

For many years, insecticides have been the most successful means of destroying mosquitoes and other insects that are disease vectors. In recent years, however, the toxic effects of these chemicals have become of increasing concern.

Last year a NIAID grantee, Dr. George B. Craig, Jr. at the University of Notre Dame, was awarded a patent for his new mosquito sterilization technique. Dr. Craig's patent describes a method of sterilizing female mosquitoes with a hormone extracted from the accessory gland of male mosquitoes. The patent also details the method of extracting and purifying this hormone, which is called "matrone." Sterilization of females, which is lifelong, can be accomplished either by injection or by feeding. The hormone can also be added to the food of mosquito larvae or sprayed on surfaces where young mosquitoes feed.

## Schistosomiasis

Since treatment is not satisfactory and control measures are inadequate, many scientists consider schistosomiasis to be one of the three great parasitology problems of the world today. In seeking new avenues of treatment, scientists supported by NIAID and the U.S.-Japan Cooperative Medical Science Program, administered by NIAID, observed recently that Philippine field laborers, repeatedly exposed to schistosome infection, show evidence of having developed a strong immunity to superinfection. This observation by Dr. Mariano G. Yogore, Jr., Robert M. Lewert, and Alfredo T. Santos, at the University of Chicago and in Leyte, Philippines, provides encouragement to other scientists seeking to find an artificial means of immunization (vaccine) to protect against the disease.

## Toxoplasmosis

In 1970 NIAID scientists and grantees identified the domestic cat as a possible reservoir of the parasitic disease toxoplasmosis. Now NIAID's Dr. Gordon Wallace has reported that in the South Pacific two species of the common filth fly are capable of contaminating human food with infectious forms of the toxoplasma parasite, picked up from the dried feces of infected cats. This finding may provide scientists with a link between human toxoplasmosis infection and the domestic cat. (The Center for Disease Control suggests that litter boxes be changed frequently to avoid accumulation of potentially infectious soil.)

# GENERAL MEDICAL SCIENCES

Several sciences basic to medicine and clinical disciplines important to the Nation's health effort were advanced during the year.

## BASIC and HUMAN GENETICS

Human genetics is a field of intense scientific interest and importance. It deals with all aspects of inherited variability within the human population, but particularly with the understanding, diagnosis, prevention, and treatment of diseases classified as "inborn errors of metabolism." The number of diseases recognized as genetically transmitted or with a significant genetic component is very large. Many genetic disorders are tragic in that they frequently strike the young and often lead to extensive hospitalization or institutionalization. Among the more common inherited disorders are cystic fibrosis, sickle cell anemia and several forms of deafness and blindness.

Other diseases have a complex genetic basis and appear to spring from the simultaneous inheritance of a number of different genes, or from a combination of genetic and environmental factors. These include such important diseases as diabetes, rheumatoid arthritis, arteriosclerosis, high blood pressure, and coronary artery disease. Almost nothing is known of genetic and molecular mechanisms responsible for these complex "polygenic" and "multifactorial" diseases.

A grantee of the National Institute of General Medical Sciences, (NIGMS), Dr. R. C. Elston at the University of North Carolina, recently obtained statistical and biochemical data supporting a polygenic mode of inheritance for the mental disorder schizophrenia. His results indicate a strong correlation between schizophrenia and the inheritance of a component (Gm) of the blood system. These findings, if confirmed and extended, could be of significant benefit in genetic counseling and the prevention of this form of mental illness.

## Genetics of the Immune Process

The study of many genetic diseases has been made possible by the discovery of strains of animals, particularly mice, having hereditary diseases similar to those of man. At the University of Washington, Dr. Arno Motulsky and associates are using the deer mouse as a model to investigate the usefulness of bone marrow transplantation in the treatment of sickle cell anemia. This disorder results from abnormal red cell hemoglobin, the molecule which carries oxygen in the blood. Transplantation of normal bone marrow, if successful in man, could lead to production of normal hemoglobin and, even if only partially successful, might control the symptoms of this disorder. Experiments by the Motulsky group have indicated that a single injection at birth of marrow from normal adult donor mice usually functions in the genetically deficient recipient animal; the very young animals do not reject the foreign cells. In other experiments on adult mice, these investigators have corrected the blood disorder by bone marrow transplantation following X-ray treatment to lessen the rejection of the transplanted cells.

Better understanding of the human immune system and its genetic basis is essential if organ transplantation is to become a fully practical measure in the treatment of disease. Scientists at Duke University, with support from both the NIGMS and the NIAID, are investigating the causes of differences in the survival of skin, bone marrow, and kidney grafts. These studies, led by Dr. Bernard Amos, include graft recipients and donors from the same families as well as unrelated donors and recipients. The Duke group has contributed importantly to the discovery that a major genetic locus (designated HL-A for "histocompatibility locus-antigen") directs the synthesis of antigens (cellular substances responsible for the rejection of foreign cells and tissues).

## Research on Chromosomes

Much useful information for the study and diagnosis of genetic disease has come from a process of photographic analysis of chromosomes called karyotyping. Technical improvements beginning in the mid-1950's made it possible to learn for the first time that the normal complement of human chromosomes is 46, or 23 pairs, and that one type of mongolism or Down's syndrome is associated with an extra, 47th chromosome in the cells of affected individuals. While many improvements in the technique have been made over the years, the analysis of chromosomes has been limited largely to examination of

their number, size, and shape. Moreover, the identification of chromosomes as belonging to one or another of the 23 human pairs has been exceedingly difficult because of their similarity and small size.

New staining methods which have been developed recently promise to increase markedly the information to be gained from chromosome analysis. Because the stains are absorbed in varying degree in different regions of each chromosome, they produce a distinct banding pattern which is unique for each pair of chromosomes, making them readily distinguishable one from another. Even more important is the prospect that chemical factors responsible for the banding will provide clues to the structure of chromosomes and the nature of chromosomal abnormalities. A very important contribution in this work is that of Dr. Margery W. Shaw, a grantee at the M.D. Anderson Hospital and University of Texas in Houston. Dr. Shaw's research group recently introduced a staining modification which allows permanent staining of the cells and improves the specificity of chromosome banding far beyond that previously obtainable.

Additional improvements were made by other investigators during the year. Dr. Herbert Lubs of the University of Colorado Medical Center, Denver, who is supported by the National Institute of Child Health and Human Development (NICHD), has devised a simple, quick, and accurate method to identify each chromosome by changing the acidity of a staining agent, thereby making the unique banding of the chromosomes immediately distinguishable as well as identifying their exact length and the location of the centromere. The technique is considerably more simple and identification more positive than is the quinacrine mustard fluorescence technique used in the comparison. Chromosomes can be identified by their pattern of banding under an ordinary light microscope.

Recent population studies have led to estimates that 20 million individuals have minor but detectable differences in the length of one or more chromosomes. This frequency is approximately 10 times the estimated figure for major chromosomal abnormalities, such as XXY, XYY, autosomal aneuploidy, or translocations. The significance of chromosomal variations is unknown but may prove to be important for genetic counseling before and during pregnancy and for the diagnosis and management of patients during childhood and adult life.

## Repair of Genetic Material

Research of the repair of damaged genetic material deoxyribonucleic acid (DNA) in bacterial cells and in human cells in culture is being conducted by a group of scientists headed by Dr. Philip C. Hanawalt at Stanford University. Dr. Hanawalt's laboratory obtained the first direct evidence for a "patching" step in the DNA repair mechanism now known as the excision-repair process. The process operates on one or another of the two strands of the helix DNA and involves several enzymatic steps: recognition that the DNA is damaged, deletion of the damaged region and correct resynthesis of the deleted region (repair replication) using the opposite strand of DNA as a template. The process has been shown to occur in many different cell types, ranging from the simplest living

cells, the mycoplasmas, to human cells. The medical significance of this finding is illustrated by the research of other geneticists, who have shown that the inability to repair DNA leads to malignancy in the case of the hereditary disease Xeroderma pigmentosum. Patients with this disease lack an enzyme needed to carry out repair of damaged DNA. Their cells therefore cannot repair DNA damage that is caused by the ultraviolet rays of sunlight. Hence, they are extremely sensitive to sunlight and develop cancers on exposed areas of skin. Dr. Hanawalt's research may throw light on the processes involved in this disease.

Most recently the Stanford group has shown in bacteria that separate enzyme systems are involved in the normal replication of DNA and in the repair replication of damaged DNA. At least two different DNA polymerases operate in the repair of short segments and long segments of damaged DNA, respectively. One of these, the DNA polymerase discovered by Dr. Arthur Kornberg, seems to act in repair of short segments. In a mutant bacterial strain lacking the Kornberg enzyme the repair function is carried out by the enzyme ordinarily used for the repair of long segments.

A further study by the Stanford group of repair replication in cancer cells (HeLa cells) has revealed that long segment DNA repair does not take place. Thus, it is possible that these mammalian cells have only the short segment repair enzyme, which cannot substitute for the long segment enzyme. Another possibility is that the HeLa cells may have lost the ability to synthesize the enzyme that repairs long segments of DNA.

Every human obtains some of his genetic characteristics from each of his parents. Each person has 23 pairs of chromosomes, one of each pair having been received from each of his parents; as he has children, one chromosome of each pair is in turn passed to his offspring on a random basis. The fact that this distribution is random contributes in large part to the genetic variation and individuality of each child. However, during the formation of the sperm and the egg, there is involved an additional process called "genetic recombination," in which small segments of the DNA intermix and interchange between the chromosomes of each pair. This important process contributes further to the individuality of the offspring, and to the maintenance of genetic variation within the species. The process of cutting and fitting the various segments of DNA together requires special enzymes capable of joining the DNA molecules together.

The enzymatic basis for genetic recombination is the focus of research by Dr. I. R. Lehman, another grantee of the NIGMS at Stanford University School of Medicine. One result of Dr. Lehman's work is the discovery of a DNA joining enzyme, called "ligase," which has the ability to seal breaks or nicks in the "backbone" of the DNA double-helix. Most recently, Dr. Lehman has shown that the ligase operates in the process of genetic recombination in bacteria. There are indications that the enzyme may be required for the replication of DNA and duplication of the chromosomes during the process of cell division. This first involves the synthesis of

relatively short DNA segments by DNA polymerase, followed by their joining together by the DNA legase. In addition, Dr. Lehman reports that DNA joining activity has been detected very recently in certain tumor-producing ribonucleic acid (RNA) viruses and may therefore be of significance in cancerous processes.

A constellation of enzymes and other proteins is believed to interact with DNA, including the enzymes active in its replication and repair. Others, such as the RNA polymerases, are responsible for translating the genetic information encoded in the DNA into messenger RNA, the actual template for protein synthesis. Depending upon the state of a cell and its needs, some DNA segments are active in protein synthesis while other segments are "turned off." The latter control is attributed to a group of regulatory proteins called "gene repressors" that bind to specific regions of the DNA and prevent the use of information in these regions for protein synthesis.

Only a relatively few proteins which interact specifically with DNA have been identified, and many more remain to be discovered. A significant development, therefore, is that of NIGMS grantee Dr. Bruce Alberts at Princeton University and his associates. The scientists were able to find and purify a protein which had previously been postulated to be the product of a particular virus gene, but which could not be isolated. It now appears that this protein functions in DNA replication by loosening up the double-stranded DNA helix and making it more accessible to other enzymes involved in replication.

## Viral Correction of Genetic Defects

Dr. Robert Weisberg, and others of the Laboratory of Molecular Genetics, NICHD, have been studying the action of DNA of certain bacterial viruses that are known to recombine with the chromosome of the host cell. When this recombination occurs, the genetic material of the virus will be inherited by all the decendants of the original cell. The viruses provide an important mechanism of genetic transfer and exchange.

In infected cells, the virus DNA usually has a unique chromosomal location because the virus-cell recombination normally occurs at a specfic site in each DNA. The investigator has been able to isolate virus cell recombinants in which the virus DNA occupies abnormal locations in the chromosome of its host. Strains have been constructed in which selected host cell genes have, by genetic fusion, been placed under the control of the virus amplifying their normal action and facilitating the isolation of the proteins they determine.

Integration and excision of viral DNA is known to change the physiology of the host organism. These viruses provide an important mechanism of genetic transfer and exchange in the microbial world. An understanding of control mechanisms operative in these easily studied microorganisms has relevance to an understanding of regulatory elements in higher organisms.

Knowledge gained in this area may have ultimate application in correction of human genetic disease by making insertion of selected genetic material to a specific site on the human chromosome theoretically possible.

## CELL STRUCTURE AND FUNCTION

The essential life processes of the body are conducted within the billions of individual cells of which it is composed. Each cell is separated from its external environment by a cell membrane. For many years scientists have studied the cell membrane to discover how it controls the passage of water, salts, foodstuffs, and waste products into and out of the cell. Although the cell membrane is important in the control of the chemicals that enter and leave the cell, it is also a barrier to the passage of larger molecules, such as hormones, which modulate and control many of the essential life processes.

### Regulator Substances

Scientists have conducted many studies attempting to elucidate how the hormones act on the cell to regulate its internal metabolism. It is now known that these hormones impinge on special target sites on the cell membrane and cause the release of a second chemical messenger within the cell, called cyclic AMP. For this work Dr. Earl Sutherland of Vanderbilt University with support from NIAMD and NHLI, won the 1971 Nobel Prize in Physiology and Medicine. Dr. Sutherland's current work, which is supported in part by NIGMS, is concentrating on the role of cyclic AMP in the regulation and control of body processes. It has been found to play a protective role against undue stress on the heart muscle, and there is tentative evidence that excessive amounts of cyclic AMP in the liver may cause the overproduction of glucose, as in diabetes. Abnormally high levels of cyclic AMP have also been found in a major form of mental illness known as manic-depressive psychosis. Dr. Sutherland has recently discovered a chemical closely related to cyclic AMP called cyclic GMP, which is widely distributed throughout the animal kingdom, but whose specific function is still being explored.

At the University of Washington, NIGMS grantee Dr. Earl W. Davie and his co-workers have shown that cyclic AMP has an important role in the synthesis of RNA, indicating that it may aid in controlling the synthesis of the cell's proteins and enzymes. Pursuit of this finding may provide clues as to how hormones act through cyclic AMP to control life processes.

### Membrane Factors

Dr. Gordon Tomkins at the University of California, San Francisco, is studying chemical factors which stimulate cells to grow and divide. Most cells, during most of their life, are in a resting--or no-growth-condition. Dr. Tomkins has found evidence that a certain chemical substance called "ppGpp" (guanosine tetraphosphate) is continuously produced within the membrane of resting cells and apparently mediates the no-growth state. Since ppGpp could not be found in the cells during rapid growth and division following their exposure to a cancer

virus, Dr. Tomkins' findings have important implications for the area of cancer research.

At the University of Colorado, Dr. Adolph Abrams and his colleagues have discovered a new and novel protein within cell membranes which they have named nectin. They have shown that nectin acts as a specific attachment site for an enzyme called adenosine triphosphatase (ATPase) which contributes to the transport of potassium molecules and amino acids into and out of the cell.

Other NIGMS grantees, Drs. Rosemary Almendinger and Lowell P. Hager, of the University of Illinois, are studying proteins called colicins which are toxic to certain cells. Their research has shown that colicins attach to, but do not penetrate, the outer membrane of target cells. They have learned that the toxic effect of colicins is exerted indirectly, being mediated through the cell membrane by another agent which signals the release within the cell of a destructive enzyme.

## Cell Structure

The role of ribosomal RNA in cell structure has been a mystery since the early 1960's when the concept of two types of RNA (messenger and ribosomal) became generally accepted. An NIAID grantee, Dr. Julian Davies, and his group at the University of Wisconsin have now reported evidence that specific stretches of ribosomal RNA may be instrumental in conferring drug resistance on an organism. The Wisconsin team reassembled ribosomes from components taken from strains of the bacterium E. coli that were resistant and sensitive to the antibiotic kasugamycine. The investigators found that one portion of a ribosomal subunit differed considerably in the resistant and sensitive strains. In the sensitive strain, one of the bases in the fragment had been chemically modified (methylated) while in the resistant strain it had been left alone.

The investigators suggest that the mutation that confers genetic resistance to kasugamycin affects a gene for a methylating enzyme. When the gene functions normally, the enzyme is made, the RNA base is methylated and the bacterium is sensitive to the antibiotic. In the resistant mutant, the enzyme is faulty, methylation does not occur, and the organism is resistant.

## Enzyme Studies

For many years, viruses were believed to consist of little more than a nucleic acid core wrapped in a protein outer coat. Enzymes necessary for virus reproduction were thought to be manufactured by the cell only after infection, as directed by the virus, or to be borrowed from the cell's normal machinery. Recently, however, investigators have been detecting an increasing number of enzymes within virus particles (virions) themselves.

In some cases, discovery of a virion-associated enzyme provides clues to mechanisms of viral replication or is otherwise of special interest.

NIAID's Dr. Lois Ann Salzman during the past year demonstrated DNA polymerase activity in association with a highly purified preparation of Kilham rat virus -- a small, single-stranded DNA virus important in cancer research. The newly detected enzyme may play a part in replication of viral genetic material -- an essential step in manufacture of new virus particles. The enzyme may be coded for by the viral DNA or may be a cellular polymerase which associates with the virion during maturation.

In a similar series of investigations, an NIAID grantee--Dr. Robert W. Simpson of Rutgers University -- and his associate reported finding RNA-dependent RNA polymerase activity in association with particles of each of 9 tested influenza virus strains of human, avian, and porcine origin.

It is possible that the RNA polymerase activity detected in the influenza viruses merely represents enzyme molecules fortuitously trapped in virus particles during their manufacture, but the investigators have preliminary evidence that the polymerase is essential for initiation of productive infections by the virus strains studied.

Another NIAID grantee, Dr. David Baltimore, has followed up on his discovery of last year that RNA-dependent DNA polymerase activity could be found in the virions of certain RNA tumor viruses. He and his group at the Massachusetts Institute of Technology have now elaborated on the enzyme's mechanism of action. They have found that reverse transcriptase -- the name given activity of the RNA-dependent DNA polymerase -- requires an RNA primer to initiate the synthesis of a DNA chain which is bound to the RNA primer. This finding was made in experiments using exogenous templates. The investigators speculate that when the endogenous tumor virus genome is the template, small RNA's, present in tumor virus particles, may act as primers.

## PHARMACOLOGY AND TOXICOLOGY

Support of research in pharmacology and toxicology is providing better understanding of the action of drugs in the human body. Ongoing research supported by NIGMS deals not only with mechanism of action and the fate of drugs in the body but also with modifications in activity resulting from the simultaneous administration of other drugs or from chemicals taken in from the environment.

## NEW DRUG USE

Dr. Thomas R. Tephly at the University of Michigan made an important contribution to treatment of the inherited condition called acute intermittent hepatic porphyria. This disorder, characterized by severe abdominal pain, is associated with excretion of an abnormal pigment in the urine. It can cause lasting liver damage and frequently is fatal. Although several drugs may be used for relief of the pain, none has been effective against the underlying problem, an inability of the patient's liver to complete the synthesis of heme. Heme is the important iron-containing component of hemoglobin and other proteins that facilitates transport and utilization of

oxygen by the body's tissues. The incomplete synthesis of heme leads to an accumulation of an intermediate chemical toxic to the body. Dr. Tephly has learned that a commonly used drug, PABA (para-amino-benzoic-acid), when combined with certain cobalt compounds, causes the toxic substance to be removed harmlessly from the body. Thus for the first time there is a promising method of treating this potentially fatal disease. For this research, Dr. Tephly was awarded the 1971 John Jacob Abel award in Pharmacology.

Because of the innumerable developments and advances in drug therapy, patients often receive several potent drugs simultaneously. On the average, a patient during a single hospitalization is given 10 different drugs. In many instances, one drug may counteract or accentuate the action of another. One example of the way in which a drug may impinge upon another is to accelerate or retard its absorption from the digestive tract, causing either too high or too low a concentration of the drug in the bloodstream.

A problem under investigation by Drs. Daniel Azarnoff and Aryeh Hurwitz at Kansas University is the ability of commonly used antacids to alter the absorption rates of orally administered drugs. These NIGMS grantees have found that antacids containing either magnesium hydroxide or aluminum hydroxide markedly lower the blood levels and therapeutic effectiveness of antibiotic sulfa drugs and of the pain-relieving drug quinine. The investigators also have found that these antacids decrease the intestinal absorption rate and the blood levels of phenobarbital, a sedative drug, thus delaying the onset of sleep.

## CLINICAL SCIENCES

Research directed at methods of improving the care and enhancing the recovery of injured and ill patients resulted in basic discoveries during the year.

### TRAUMA

Many seriously injured patients suffer from shock, a poorly understood syndrome which usually follows a severe injury and may be fatal. It is therefore important to diagnose and measure the severity of shock in all accident victims. Shock is usually treated by the transfusion of blood or related fluids, but results are variable, and scientists are probing more deeply to discover underlying subtle factors that may be involved.

Dr. Thomas Shires and his associates at the Parkland Memorial Hospital Trauma Center in Dallas have found that the amount of sodium present in red blood cells rises with the onset of shock, and that the degree of elevation correlates very closely with both the magnitude and duration of shock. Their discovery, if confirmed, may have implications for improved early diagnosis of shock and for monitoring the effectiveness of therapy.

#### Burn Therapy

Some 70,000 Americans each year are admitted to hospitals with

severe burns and of these victims about 12,000 die. A frequent complication of burn injury and a reason for the high mortality rate is the rapid and relatively enormous loss of water from the body following the destruction of the protective skin surface. The evaporation is accompanied by a rapid loss of body heat, which further disrupts the patient's vital functions. In research to control the loss of water from burned surfaces, Dr. Carl Jelenko III, an NIGMS grantee at the Medical College of Georgia, recently found that an oily substance called ethyl linoleate is present in normal skin but not in burned skin, and appears to function in the retention of water. He then demonstrated that commercail preparations of ethyl linoleate when sprayed on burns reduced water loss by as much as 50 percent compared to untreated burns. It is thus possible that in the near future ethyl linoleate will be accepted as a valuable adjunct to burn therapy.

### Color Radiography

A significant accomplishment in diagnostic radiology during the past year has been the development of a color imaging process which promises great improvement in interpretation of X-ray films. Scientists of the Rand Corporation developed the procedure, which converts the variations in shades of gray found in the conventional X-ray negative into colors and hues. Full use of the information in black and white radio graphic images is limited because the human eye can distinguish simultaneously only about 15 shades of gray, while a standard X-ray negative contains hundreds of shades of gray. When the image is converted to color a vast amount of detail not previously seen becomes recognizable.

## DEVELOPMENT AND AUTOMATION OF CLINICAL LABORATORIES

Many of the benefits of medical research reach the patient through the clinical laboratory which serves as a conduit between the biomedical sciences and clinical medicine. With the growth of biomedical knowledge, clinical laboratory technology must be continually improved and extended. Automation of clinical laboratory procedures is needed to provide the fullest range of diagnostic services to the patient at lowest cost. For these reasons NIGMS encourages and supports basic and applied research leading to the automation of clinical laboratories.

### CELL ANALYSIS

A new area of importance is the development and automation of instrumentation to separate and analyze different kinds of cells and to measure or detect changes in their characteristics which may be indicative of disease. Some success has been achieved, as in the partial automation of red and white blood cell counters, but much remains to be done. For example, there are at least six structurally and functionally different types of white cells, none of which can be singled out for separate study in flow-through analytical systems.

Promising research on automated cell analysis is being

conducted by Dr. Leonard A. Herzenberg at Stanford University. Dr. Herzenberg has developed an instrument which automatically separates cells on the basis of their fluorescent properties. The cells are suspended in solution with a fluorescent dye or stain which is absorbed more readily by one type of cell than by another. The cells then pass into a chamber where the stained ones are defected electrically into separate containers, depending upon the strength of their fluorescence. Dr. Herzenberg has used the instrument to separate young red cells known as reticulocytes; in addition, the characteristics of cells recovered have faithfully reflected several different kinds of anemia.

It is believed that the instrument can now be modified and improved to separate the complex mixtures of cells found in such organs as the spleen, bone marrow, and lymph nodes. Another possibility is that it will readily separate cancer from noncancerous cells, and also fetal cells from maternal cells in the analysis of amniotic fluid samples. Very intriguing, too, is the prospect that it can be used to sort out young cells, in their earliest developmental stages, and the same kinds of cells in their later, mature form, all of which would have important implication for studies on the human aging processes.

## ENZYME ANALYSIS

An area of continuing interest is that of enzyme analysis. Hundreds of enzymes are synthesized in the body to carry out the processes of metabolism and in one way or another enzymes are involved in every human disease. For example, the enzyme lactic dehydrogenase (LDH) is normally contained in muscle tissue but is released into the blood stream following a heart attack. The analysis of LDH in a patient's blood thus helps the physician know that he is dealing with a heart attack rather than some other condition with similar symptoms.

Dr. Harry L. Pardue, an NIGMS grantee at Purdue University, is developing an automated system to analyze enzymes in body fluids rapidly and accurately. He has wedded the capabilities of a small spectrophotometer to a computer. The spectrophotometer measures and reports to the computer the rates at which enzymes under study react with reagent chemicals; the data are then calculated and the amount of the enzyme in the sample is expressed directly as a numerical readout. The unique feature of Dr. Pardue's instrument is modification of its circuitry for feedback control, which permits an analytical accuracy of not less than 99 percent, compared to 80-85 percent accuracy with conventional equipment. Dr. Pardue has successfully studied the enzyme LDH and others, including alkaline phosphatase and glutamic oxalacetic transaminase (GOT). Abnormal levels of alkaline phosphatase in the blood may signal leukemia; GOT is frequently associated with injury to the liver. Dr. Pardue has begun to expand the system's capacity from single samples to the simultaneous analysis of multiple samples, and automation of all sample handling procedures.

## COMPUTER RESEARCH AND TECHNOLOGY

The computing needs of NIH research scientists and administrators are diverse. Consequently, the Division of Computer Research and Technology (DCRT) Computer Center Branch actively searches for the most advanced but economical techniques for meeting those needs. An IBM 370-165 computer was added to the central machine complex during August of 1971. It replaced 2 of the 4 processors previously in the Computer Center. The 2600 registered users of the Computer Center account for more than 3500 jobs processed daily. The number of remote terminal users who can communicate directly with the machines at any one time has increased to 130.

## MEDICAL COMPUTING

The Data Management Branch has developed a way of automating the writing of computer report programs for some users. By employing an interactive terminal system, the computer prompts the user to answer a series of questions. His answers are then translated into the statements of a standardized programming language, which may be one of several but must be chosen beforehand. The program acts upon his data and furnishes him with reports. A significant amount of programming time is saved, and the user maintains close interaction with his data.

### Cancer Data Analysis

Led by Dr. John Cooper and Dr. Norbert Page, a group in the Office of the Associate Director for Carcinogenesis of the National Cancer Institute has been compiling a massive amount of data dealing with the carcinogenic effects of chemicals on animals. Data is garnered from outside contractors, academic and private.

All of the information is entered into computer files from specially designed forms. Once the files are set up, specific reports can be generated describing such things as types of tumors, chemical agents which are active, and kinds of animals in which tumors grow easily. In addition computer programs are being written so that statistical analyses can be performed. From this endeavor it will be possible to produce curves of animal survival and to correlate different elements of the data into meaningful reports.

The system and computer programs were designed by Mary Linhart and Larry Martin of the Data Management Branch at DCRT.

### Mass Spectra of Unknown Compounds

Chemists face the problem of identifying unknown substances, often a tedious and time-consuming process. One of many methods used is mass spectroscopy. A sample of the unknown compounds is inserted into the mass spectrometer. There, under the onslaught of high energy electrons, it is broken down into fragments, each of which is characterized by a peak in the spectrum. The combination of these peaks yields a unique "fingerprint" for the compound.

The tedious aspect of such a search involves matching the mass

spectrum of the unknown with a file of known compounds. Manually, this task is laborious. With a computer, however, it can be done almost instantaneously. Dr. Stephen Heller, a chemist with DCRT, has collaborated with Drs. Henry Fales and G. W. A. Milne of the National Heart and Lung Institute. They have designed a system which searches rapidly and interactively for spectrum peaks, molecular weight and molecular formulae. The chemist, using a remote terminal which is attached to the computer via telephone lines, types in the information he has on the peak locations and intensities in his unknown. This information is matched against a data base of over 8,100 spectra compiled by Professor Klaus Biemann at the Massachusetts Institute of Technology. The matching occurs peak by peak so that the user can not only identify his original compound, but obtain information on interesting related compounds which have a similar pattern.

## Nuclear Magnetic Resonance (NMR) Studies of Proteins

Nuclear Magnetic Resonance is a technique used for studying chemical structure. It employs an intense magnetic field and allows characteristic spectral properties of molecules to be observed. The technique has been applied to proteins by Dr. Jack Cohen of DCRT and Dr. Alan Schechter of the National Institute of Arthritis and Metabolic Diseases. They have been studying the individual hydrogen atoms of histidine, an amino acid contained in a number of enzymes. Enzymes are proteins which perform most of the metabolic functions of the living cell. Using a statistical (least-squares) computer program, written by Richard Shrager of DCRT, the NMR data can be analyzed and the results can be displayed on a cathode ray tube.

Under normal conditions, a protein chain is folded in a certain way and this gives the enzyme its characteristic activity. However, enzymes can be denatured and become inactive. Controversy exists on whether the unfolding of the protein which is characteristic of denaturation occurs in one step or if the molecule goes through several intermediate states.

The investigators were able to follow the behavior of the histidine residues in an enzyme called nuclease and show that in at least one form of denaturation there is evidence for the existence of intermediate states during the unfolding process.

A computerized molecular display system has been set up by Richard Feldmann and Stephen Heller. Using structural data derived from X-ray crystallographic studies on such large molecules as nuclease, the researchers have been able to display a 3-dimensional molecular model. They can view all or part of a molecule, rotate it and in this way correlate their findings from NMR studies with what is known of the crystal structure of the protein.

## Modeling and Curve Fitting

MLAB, short for modeling laboratory, is a computer tool used for experimenting with and evaluating mathematical models. The system has been designed by Gary Knott and Douglas Reece of the DCRT

Heuristics Laboratory for use by investigators interested in conceptual mathematical analysis, mathematical modeling and displaying the results of their mathematical operations.

The system is interactive. That is, the user sits down at a teletype (which can be remotely located) and works with the computer by "conversing" back and forth. The statements in MLAB are simple, direct and, therefore easy to It has been designed to do away with the necessity for any user programming.

In modeling, one takes experimental data and either formulates an equation to explain them, or tries to evaluate certain unknown parameters. MLAB allows a user to test the validity of his mathematics or to compute suitable parameter values which cause his model to best fit his data. The on-line graphics display shows him how good his fit was and my help him to study his model further.

Hard copy of any of the displayed pictures can be produced by the system.

The system has been used by chemists studying equilibrium phenomena, and bond interactivity in chemical compounds. Physicians employ it for studying absorption of radioactive tracers, neural transport mechanisms and drug interactions.

## Fat Metabolism and Mathematical Modeling

Fatty acids are carried in the blood by a protein called serum albumin. An understanding of how the fat is transferred from serum to cell has important applications in the treatment of obesity, coronary heart disease and other disorders related to the metabolism of body fat. We know that the exchange is effected by a series of complex chemical equilibrium reactions.

Analyzing the data from such chemical equilibria and using new mathematical models and computing methods, John Fletcher and J. Douglas Ashbrook of the Laboratory of Applied Studies have re-examined earlier concepts of fatty acid uptake. They have been able to demonstrate hitherto unrecognized classes of binding sites between molecules and exchange rates which are different from what was previously supposed. These models have contributed to the understanding of fat transfer mechanisms.

# CHILD HEALTH

Research directed at improving the health of children and of finding ways to prevent and treat diseases of childhood achieved substantial progress during the year.

## MENTAL RETARDATION

Dr. Rick Heber, Professor of Education and Child Psychology at the Mental Retardation Research Center at the University of Wisconsin, Milwaukee, has found that the intelligence quotient of children from

a low intelligence population can be increased markedly through a program of early and concentrated intervention.

The population group in this longitudinal study consisted of mildly retarded persons without important related physical problems, who were for the most part not identified and serviced by community, social, or rehabilitation agencies. The research was supported by the National Institute of Child Health and Human Development (NICHD).

The area selected for the studies was a residential section of Milwaukee which had the lowest median family income, the greatest population density per housing unit, and the most dilapidated housing in the city. This area yielded a much higher rate of mental retardation among school children than any other section, yet the majority of persons in most slum areas are not retarded, and the children reared there develop and learn in a relatively normal fashion.

The investigators found that maternal intelligence was the most reliable single indicator of the level and character of intellectual development of the children. Although mothers with intelligence quotients (IQ's) of less than 75 made up less than half of the total group of mothers in the study, they accounted for about four-fifths of children with low IQ's. (Normal intelligence is indicated by an IQ of about 100). The investigators also showed that the lower the mother's IQ the greater the possibility of their children's scoring low on intelligence tests.

These factors suggested the need for a total family approach to rehabilitation and prevention. Adult women with Wechsler Adult Intelligence Scale (WAIS IQ) scores below 75 and with a newborn child were randomly assigned to an experimental comprehensive family stimulation program and control group. Parents and all children residing in the home are involved. Mothers receive mothering, child-care and homemaking training, motivational reeducation, remedial education, vocational counseling and occupational training. Infants beginning at a mean age of two months were enrolled in a carefully prescribed program designed to facilitate intellectual, academic, social and occupational development. Children participated on an 8-hour per-day, seven-days-a-week basis throughout the year. The adult staff-to-child ratio was 1-to-1 during infancy and was decreased as the children became older.

At the present time, the 25 experimental group children, who now average about 5 years of age, are superior in all measures of development, and particularly in language, to the control group. The discrepancy between the two groups, about 35 points in IQ, seems to be increasing with age. The experimental group now shows an acceleration in rate of development and has a mean IQ of 128, while the control group is declining from its average performance in infancy.

The experimental group mothers, being both retarded and disadvantaged, had no prior work history. About 50% of the original mothers enrolled are now employed, while a significant proportion

of the remaining are involved in training or educational programs.

The dramatic differences between the two groups, if maintained through the early years of formal schooling, could have a substantial impact on practices followed in rehabilitating and educating mildly retarded children.

## Phenylketonuria

Phenylketonuria (PKU) is an inborn error of metabolism which when untreated can produce mental retardation. Those afflicted with the disease have an IQ which deteriorates within the first few years of life to usually 50 or less. (Normal IQ level is 90-110). The disorder is also typified by hyperactivity, destructive behavior and emotional imbalance.

For years, researchers have attempted to develop an animal model of PKU that would aid in assessment of the relative value of various treatment methods and aid in study of the mechanism by which a specific metabolic abnormality causes brain and behavioral anomalies. NICHD scientists Dr. Arnold Andersen and Dr. Gordon Guroff now have successfully produced an accurate model of PKU in the rat. Using a five-part methodology in their experimentation, they have eliminated discrepancies which caused prior studies to be invalid. Andersen and Guroff used three groups of rats. A group of 28 newborn rats (Group I) were injected for 21 successive days with saline solution, to serve as controls in the study; phenylalanine in saline solution was administered Group II, and p-chlorophenylalanine (PCP) supplemented with phenylalanine (Phe) in saline solution was administered to Group III. This latter group, consisting of 13 rats, exhibited behavioral, biochemical and neurological symptoms characteristic of PKU during the treatment period. The rats developed hyperphenylalaninemia with normal or lowered blood tyrosine, and as a direct result of the inhibiting effect of PCP also showed absence of phenylalanine hyroxylase activity in the liver. Evidence of heightened cerebral excitability was revealed, and hyperactivity and a deficit in learning capabilities was shown by testing. Signs of aggression were also present. Physiological characteristics of PKU, abnormal myelinization and undersized brains, were detected at autopsy. With the Andersen-Guroff methods, scientists will now be able to probe for possible preventive measures and improve treatments for PKU victims.

## HEMOPHILUS INFLUENZAE

Hemophilus influenzae type b is a bacterium that causes several serious diseases in infants and children, the most common being meningitis. Based upon the number of deaths reported to the Center for Disease Control, and upon statistics derived by Dr. John Robbins and a team of workers of the NICHD from hospital records of Mecklenberg County, North Carolina, it is assumed that 12,000 to 15,000 infants and children contract Hemophilus influenzae type b meningitis annually. Approximately 20 to 50% of victims of this disease who recover after treatment with antibiotics suffer

permanent central nervous system deficits including convulsive disorders, deafness and mental retardation; these figures refer only to those with meningitis, one of the several diseases caused by Hemophilus influenzae type b.

Newborns possess an immunity to this disease from serum antibodies acquired from the maternal circulation before birth. The immunity possessed by adults is developed after six years of age.

Experimental approach to preventive immunization has been initiated by selection of gastrointestinal bacteria which possess a polysaccharide covering structurally similar to the covering of Hemophilus influenzae type b. Using adaption of a previous technique, several non-pathogenic Escherichia coli (common inhabitant of the intestine) have been isolated and the chemical composition studied. Preliminary work in animals indicates that a simple preventive immunization program may be possible by feeding an infrequently found variant of this common bowel organism to newborn humans. This feeding procedure may therefore accelerate the time of appearance of adult immunity to diseases caused by this organism.

## RESPIRATORY DISTRESS SYNDROME

A rapid chemical test devised by an NICHD-supported investigator is now being used in medical centers throughout the country to determine whether a premature infant will be at risk for respiratory distress syndrome (RDS) at birth.

Dr. Louis Gluck at the University of California, San Diego, has measured the maturity of the fetal lung by comparing the ratio of two lung phospholipids, lecithin and sphingomyelin, present in amniotic fluid. Until about the 30th week of gestation the concentration of sphingomyelin in the fluid is usually higher than that of lecithin. The lecithin concentration builds up slowly until 35 weeks, then as the lung matures increases sharply to levels more than twice that of sphingomyelin. The change in the ratio signifies that pulmonary maturity has advanced to a stage that permits extrauterine breathing by the newborn to proceed normally. In several hundred "high risk" pregnancies studied, the test proved accurate in evaluating pulmonary maturity and therefore the likelihood of the baby's developing RDS.

About 25 percent of infants born prematurely show signs of respiratory distress syndrome and over 20 percent of these, an estimated 25,000, die each year from it. Clinical application of this new method of diagnosis is expected to reduce the occurrence of the RDS.

## INDIVIDUAL DIFFERENCES IN TWINS

The temperament and attention span of normal one-year-old children may be predicators of later cognitive abilities, according to results of an investigation involving 56 pairs of 12-month-old twins. NICHD-supported investigators, Drs. Adam P. Matheny and Ann M. Brown at the University of Louisville School of Medicine, observed

that one twin typically was clearly more active physically than his co-twin. The more active twin learned to walk sooner and had temperamental outbursts more frequently. The other twin, however, had a longer attention span, was less active physically, and evidenced persistency and placidity.

In follow-up studies three years later on the same children, Drs. Matheny and Brown found that the less active twin possessed significantly better cognitive abilities compared to his active mate. These results suggest that attention span is associated with higher intelligence quotient. Attentive co-twins also persisted longer at manipulative play and developed dexterity in tasks involving spatial relations, attention to figural-ground contrasts, and discrimination among the properties of objects.

Although parental characteristics are known to influence infant behavior, the scientists believe that certain intrinsic characteristics have a pervasive influence on development in spite of very similar life experiences of twin pairs from early childhood. In future studies, the researchers hope to define some of these intrinsic characteristics to better understand human behavior patterns

## POPULATION

Research of several kinds contributed during the year to increased knowledge of population problems and suggested pathways to solutions.

### THE FEMALE REPRODUCTIVE SYSTEM

Two important findings concerning the female reproductive system were reported this year by Dr. Arpad Csapo and colleagues at the Washington University School of Medicine, with support by the National Institute of Child Health and Human Development (NICHD).

One study, conducted in Finland, dealt with the temporal relationship of the corpus luteum to pregnancy maintenance. It confirms that the corpus luteum, a progesterone-producing structure which forms on the ovary after ovulation, is a feasible target organ for fertility control research.

Dr. Csapo studied Finnish women hospitalized for elective pregnancy termination. He found that in women pregnant for approximately seven weeks removal of the corpus luteum resulted in decreased plasma progesterone levels and was followed by miscarriage five days after the surgery. In women pregnant for approximately nine weeks, removal of the corpus luteum resulted only in a transient lowering of plasma progesterone levels and miscarriage did not occur.

These results indicate that maintenance of pregnancy is dependent on corpus luteum function for approximately nine weeks, after which the placenta begins to secrete sufficient progesterone to maintain pregnancy in the absence of the corpus luteum.

## Oviduct Studies

The second study dealt with the physiology of the rabbit oviduct, in which he discovered a "pacemaker."

Measurements were made of the electrical activity of the various anatomical segments that comprise the oviduct (the tube in which fertilization occurs). Between the ampulla and the isthmus, two consecutive sections of the oviduct, is a specialized area known to be important in controlling the movement of ova and sperm through the oviduct. The scientists found that electrical discharges are most frequent from this specialized area and that it acts as a pacemaker, controlling muscular activity in the oviduct. The degree of electrical activity in the pacemaker area correlates with pressure inside the oviduct. If drugs could be developed to change the pacemaker function, they would influence the passage of ova and sperm through the oviduct and could thus exert an antifertility effect.

Dr. Louise Odor, Medical College of Virginia, in collaboration with Dr. Richard Blandau, University of Washington, NICHD grantees, has been studying the effect of hormones on cilia and ovum transport in the rabbit oviduct. She found that in rabbits in which the ovaries have been removed, the oviduct loses most of its cilia and the transport of surgically introduced ova into the oviduct is impaired. Treatment of ovariectomized rabbits with estrogen restores ciliary growth. Likewise, ova placed at the ovarian end of the oviduct (fimbrium) are transported into the duct.

In a related study Drs. Ellen Dirksen and Peter Satir of the University of California at Berkley are investigating the fine structure of oviductal cilia. They observed that at the tip of each cilium are a number of fine hairlike projections which could have an important function in grasping the ovulated egg and directing it into the oviduct. The number of cells containing cilia varies along the length of the oviduct and is greatest at the ovarian end. This particular distribution is important to ovum transport because in the ovarian end of the oviduct muscle activity is less prominent than in the other segments of the tube.

## Prostaglandins

The influence of prostaglandins (PG's) in reproductive function has been the subject of intensive research supported by NICHD. Though these body chemicals are known to disrupt corpus luteum function in a number of laboratory animals, recent studies show marked differences among species.

In hamsters, Dr. Kenneth Kirton of the Upjohn Company found that luteolysis (destruction of the corpus luteum) by PGs can be counteracted by estradiol, suggesting the PG effect is mediated via reduction of blood flow through the ovary. Estradiol improves this flow.

Dr. Roy Greep and associates at Harvard Medical School have injected the prostoglandin PGF2 alpha into pregnant rats, which

drastically reduced the production of progesterone by the corpus luteum but increased its production of another less active progestin. This quantitative shift represents deterioration in corpus luteum function. However, addition of the substance to rat corpora lutea in test tube preparations increased the production of both progestins. This indicates that the prostaglandin acts differently in living animals than in laboratory tests and careful assessment of results must include consideration of these differences.

Information on what role PG's play in primate reproduction is expected from NICHD projects in which prostaglandin analogs and antagonists are being synthesized and tested.

## New Brain-based Hormones

Ten years of research have culminated in determination of the molecular structure and synthesis of luteinizing hormone-releasing hormone, or LH-RH, which is elaborated by the hypothalamus, a portion of the brain. This hypothalamic hormone controls the release of luteinizing hormone (a hormone essential for ovulation) from the pituitary gland. This accomplishment might lead to more effective control of human fertility than possible heretofore and to development of new methods of birth control.

Dr. Andrew V. Schally of Tulane University, an NIAMD grantee, and his associates reported their accomplishment before the 1971 meeting of the Endocrine Society in San Francisco. A few months later, in the fall of 1971, the same team of scientists described studies in hamsters in which subcutaneous injection of synthetic LH-RH (a relatively simple chemical compound) stimulated release by the pituitary gland of luteinizing hormone and induced ovulation. There were no undesirable side effects associated with administration of synthetic LH-RH, which should become a useful tool in both clinical and veterinary fields for fertility induction and regulation.

## DETECTION OF PREGNANCY AND CANCER

A cooperative basic research effort between the NICHD's laboratory scientists and the Center for Population Research has resulted in one of the outstanding accomplishments of many years.

The work involved extracting, purifying, and splitting a hormone (HCG) produced by the placenta and by some cancerous tissues. The scientists then developed a method to discriminate the placental hormone from a similar pituitary hormone, resulting in a test which detects pregnancy as early as 8 days after conception (one week earlier than available research tests and 3 to 5 weeks earlier than routine pregnancy tests).

This accomplishment resulted from basic research on the hormone's structure; its clinical application was an unforeseen bonus. The scientists proposed that the structure of HCG be analyzed by an appropriate contractor, and funded by the Center for Population Research. A collaborative laboratory (Dr. Robert Canfield, Columbia University) found that HCG is composed of two dissimilar subunits,

one unique to HCG and the other found in pituitary hormones. The NICHD scientists (Drs. Griff Ross, J. Vaitukaitis, and others) then used the unique subunit to make antibodies in rabbits and devised a test using the immunologic reaction that was specific for HCG. NICHD has applied for a patent which would assure that the test would be made available to the public at the lowest possible cost.

This research also will have an important application to the treatment of cancer, in that the test can be used to diagnose certain cancers, follow their progression, and ascertain whether surgery has been successful in removing all of the diseased (hormone-producing) tissue. It will also facilitate greatly the efforts of scientists and workers in population research who study aspects of early pregnancy.

## ORAL CONTRACEPTIVES

A study by NICHD scientists has defined the menstrual cycle changes experienced by women while taking oral contraceptives (OC's) and after discontinuing their use.

During OC therapy, both combined and sequential OC preparations tend to regulate cycle length while reducing the average from 31 to 26.4 days. Differences between individuals in mean cycle length, however, ranged up to 11 days, a surprising finding. One-fourth of women whose cycle length varied less than 10 days before OC therapy experienced greater variability while taking OC's. A shift toward slightly shorter flows (but more flows per year) was demonstrated in the group of 633 women taking OC's.

After discontinuing OC therapy, a significant number of women failed to quickly reestablish their normal menstrual patterns. Average duration of flow returned to its previous value, but the interval between menstrual periods for many women was changed. These findings, coupled with increasing reports by physicians of post-pill amenorrhea (no menstruation), stress the need for further research.

The study by Dr. Alan Treloar and the late Dr. Borghild Behn of NICHD, will continue, to see how enduring the effects of OC's are on the menstrual cycle. Other effects of OC's on general health will also be reported by participants in Dr. Treloar's menstrual history survey, which has compiled menstrual histories on almost 6,000 women during the past 38 years.

### Effects of Oral Contraceptives

The Contraceptive Drug Study at the Kaiser Foundation Hospital, Walnut Creek, California, involves evaluation and re-evaluations of 10,000 women through a multiphasic screening procedure. Between evaluations, women who become in- or-out patients at the Walnut Creek Hospital facility are kept under surveillance. Based on preliminary analyses of the data, a number of results have been reported.

The blood coagulation mechanism of women using OC's shows greater activity than that of nonusers. This, in itself, may not be of pathological significance; the study will be expanded to

include measuring blood flow, a technique which might be useful in detecting an excessive tendency to blood clotting within blood vessels.

A modest increase in blood pressure has been found in OC users when compared to nonusers. The change is directly proportional to age and weight as well as a variety of other factors, but it does not appear to be related to any specific drug product.

Decreased tolerance to glucose in OC users has been confirmed, and the change is related to body weight and age. No difference in a number of measurements of pulmonary function was found between users and nonusers. OC users do not appear to be more depressed than nonusers, and the drugs appear to have a beneficial effect in alleviating premenstrual tension.

## STERILIZATION

A recent study of a suburban, middle-class California community indicated that 31 percent of the couples surveyed had undergone some form of sterilizing surgery. This seems indicative of a new trend in choice of birth control method.

As part of a Contraceptive Drug Study carried out by the Kaiser-Permanente Medical Care Program, a mail survey was conducted in a suburban area near San Francisco in 1968 to collect data on methods used to prevent conception, including surgical sterilization. Ninety-two percent of these receiving questionnaires responded, giving data on white married persons of an educational and economic level slightly above the national average.

Sterilizing operations were divided into two categories--remedial and contraceptive. Remedial operations were those intended to correct a pathological disorder; contraceptive operations were intended to prevent further pregnancies.

Among couples with the wife's age ranging from 20-54 years, 23 percent had had contraceptive operations. Over two-thirds of the operations were vasectomies performed on the husband.

The number of children the wife had borne, the couple's religion and their education were factors which influenced the prevalence of sterilizing operations.

The percentage of couples with contraceptive operations increased with the number of children they had. Hence, prior to surgery the voluntarily sterile couples had, on the average, a slightly larger number of children than others, but rarely more than four.

Sterilization with contraceptive intent was twice as common when the wife had less than a high school education than when she was a college graduate (32 percent versus 14 percent). The earlier age at which less educated women begin childbearing (and therefore complete their families) may explain the difference.

The prevalence of contraceptive operations was lowest when both husband and wife were Catholic (15 percent) and highest when neither was Catholic (25 percent). Nonetheless, contraceptive operations were found with remarkable frequency among Catholic couples who attend church regularly, according to the investigator, Dr. Nancy Phillips.

## THE 1970 NATIONAL FERTILITY STUDY

Every five years since 1955, a study has been conducted with NICHD support of the contraceptive attitudes and practices, and the fertility aspirations and experiences of American women. In 1970, Drs. Charles Westoff and Norman Ryder of Princeton University interviewed 6,752 women who have been married. Preliminary indications show results for approximately 6,000 currently married women of reproductive age.

Every fertility indicator is sharply down, including ideal, desired, and intended family size. As an example, ideal family size has dropped from 3.3 children to 2.7 in five years. In a similar study conducted in 1965, the proportions of women citing 2, 3, and 4 children as the ideal number were approximately 24, 33, and 35 percent; in 1970 they were 48, 23, and 22 percent.

An average decline of 14 percent was evident in the total number of children women aged 20-29 intended to have. It is obvious American women today want to expect to have fewer children than they did several years ago. Reasons for this are unknown but may include economic and labor market considerations. A similar change in the fertility of American women occurred in the 1920's. This trend was later reversed, illustrating that caution is necessary in interpreting short-term fluctuations.

In 1970 more couples were using highly effective birth control methods, and the investigators noted "a very substantial increase in the favorability of attitudes toward abortion...between 1965 and 1970."

Fifty-two percent of women reported that they or their husbands were currently using some method of contraception. The proportion of these couples using either the oral contraceptives or intrauterine devices (IUD's) has increased in 5 years from less than 30 percent to more than 50 percent. If these rough percentages are applied to the total U.S. population of currently married women of reproductive age, estimates would suggest that 7 million women are now using either "the pill" or IUD, 7 million married couples are using less effective forms of contraception, and 14 million couples are not practicing birth control. Some of the 14 million nonusers are sterile; some are experiencing a pregnancy or planning one; and others are not using contraception for various reasons.

The proportion of married women who had ever used the pill has grown from 26 percent in 1965 to nearly 60 percent. The study shows a peak in use of the pill in November 1969, followed by an abrupt slump over the next 6 months. According to Dr. Ryder, the drop appears to be accounted for entirely by concern over reports that the use of the pill may be harmful. In the last half of 1970 the

rise in pill use resumed at a slow rate.

The investigators also report that approximately 1 couple in 6 has been sterilized, and of these, about one-third are husbands (vasectomies) and two-thirds wives (half of them by hysterectomy and half by tubal ligation).

## Fertility Variations

A recent survey of U. S. women shows wide variation in fertility by ethnic origin.

In 1969, women 35-44 years old, most of whom had completed childbearing, had borne an average of 3.0 children. Highest fertility was among women of Mexican extraction (4.4 children) and the lowest was among those of Russian extraction (2.4), followed by Italian (2.4), Polish (2.5), English (2.8), German (3.0), and Irish (3.1). Separate computations showed black women having 3.6 children and white women 2.9.

The impact of these fertility rates on population growth can be illustrated by comparing two groups: the rate for women of Mexican extraction implies a potential doubling of this group's population in about 26 years (one generation); in contrast, the rate of all white women implies a population increase of 40 percent per generation.

Women residing in low-income areas tend to be more fertile than those living in other areas within large cities. This fact may help to explain the higher fertility of women of Spanish extraction and of blacks, a larger proportion of whom live in low-income metropolitan areas.

The relation between fertility and family income varies from one ethnic group to another, but high family income is associated with low fertility. For example, fertility of 2.4 children ever born to blacks in the highest fourth of black family incomes is not significantly different from that for whites in the highest fourth of white family incomes.

## Illegitimacy

Early results of a study of illegitimacy under the direction of Dr. Kingsley Davis, University of California, Berkeley, have underscored the importance of this phenomenon in the United States. In California in 1967, about 10 percent of all births, or 35,215, were out of wedlock. The illegitimacy rate of 27.2 births per 1,000 unmarried women aged 15-44 compares to an estimated rate for the United States of 23.9. The rate for white women in California was about twice that for white women in the U. S. as a whole. The rate among blacks was 4 to 5 times as high as among whites in California, but is closer to the national average for blacks. Forty percent of white illegitimate births and half of black illegitimate births were to women under 20. Two-thirds of white and half of black illegitimate births were firstborn children.

Illegitimacy occurs in all segments of the population but is more frequent among lower income groups. Whether this is cause or effect--or both--is unclear. It is clear that unmarried women are more likely to delay pernatal care, and their babies tend to weigh less and be at higher risk of death in infancy.

Estimates of illegitimate births are limited by 2 factors: an unknown number are recorded as legitimate and some are not recorded at all. Using estimates of the Department of Health, Education and Welfare, the rate of illegitimate births in the U.S. has more than tripled since 1940, despite the availability of modern contraceptive methods.

## AGING

Significant results were reported during the past year in a number of areas related to the aging process.

### INTELLIGENCE AND BLOOD PRESSURE

Investigators at the Center for the Study of Aging and Human Development at Duke University Medical Center have found that high blood pressure (hypertension) adversely affects the mental performance of the elderly.

Dr. Carl Eisdorfer and a team of investigators examined 202 normal volunteers, whose sex, race, and socio-economic characteristics approximate those in the Durham, North Carolina, population over a 10-year period. The blood pressure and intelligence tests were given as part of an on-going multidisciplinary longitudinal study supported by NICHD. At the start of the study, ages of the subjects ranged from 60 to 79. Subjects were evaluated for a 2-day period about every 2½ years.

Participants in their 60's and those in their 70's were separated into 3 groups according to their diastolic blood pressure. Readings of 65 to 95 were considered to be normal, those between 96 and 105 to be borderline elevated, and those above 105 to be high.

Those in the 60-69 age group had initial Wechsler Adult Intelligence Scale test scores ranging from the high 80's to the low 90's. (Normal is approximately 100). At the end of the 10th year, those who had normal blood pressure reading showed virtually no WAIS score changes. Those who had borderline elevated blood pressure increased their average score by a little over 3 points. A marked test loss of 7 points was found in those participants with high blood pressure. This decline correlated with loss in verbal performance and manual dexterity.

Among those in their 70's at the beginning of the project, none with high blood pressure completed the 10 years of the study. The WAIS scores of those with normal blood pressure had dropped by only 3 points 10 years later, while those whose blood pressure were borderline dropped almost 6 points.

## GLUCOSE UTILIZATION

Research on aging processes in man is complicated by the fact that the gradual deterioration in functional capacity in many organ systems with advancing age has not been clearly differentiated from development of certain diseases which also increase progressively with age. Yet the separation of what might be considered "normal aging" from true disease is critical in terms of assuring diagnostic accuracy and thus also in terms of proper therapy.

The disease diabetes mellitus has been intensively studied as a prime example of these difficulties. Scientists in the NICHD Gerontology Research Center's Clinical Physiology Branch have stressed for several years the necessity of using age-corrected standards for diagnosing the disease. The potential seriousness of this error has been emphasized recently by reports of potentially harmful effects of administration of oral anti-diabetic drugs to older individuals. There has thus been a renewed emphasis on insulin therapy in this age group. Very little is known, however, either about the effect of age on the metabolism of insulin or about the magnitude of the biological effects that it produces in older individuals. Recent studies have therefore been carried out on the kinetics of insulin distribution in the body, on its rate of degradation, and on its effects on the tissues.

These studies utilized the "glucose-clamp" technique developed at the Center to control blood sugar levels during insulin infusions. Complex data analysis by new computer methods was required. Several new findings emerged.

The rate of distribution of insulin into the several fluid compartments of the body is unaffected by age. Thus the theory that hormones lose effectiveness with age because of hindrances to their rapid distribution throughout the body could not be supported.

The rate of degradation of insulin also is unchanged with age. Thus the possibility that aging lowers circulating hormone levels by rapidly destroying the hormone after its release was also shown not to be true.

The effectiveness of insulin in exerting its biological actions on the tissues of the body is not decreased in older subjects. Thus insulin was shown to be normally distributed, normally degraded, and normally active in older subjects.

These studies offer strong support for the hypothesis that the primary defect which underlies the inefficient utilization of glucose in advancing age rests in the inadequate insulin production and releasing mechanisms of the pancreatic beta cells.

## PERFORMANCE OF THE HEART

Previously studies at the Center have shown that the amount of blood pumped by the heart each minute diminishes with age. Studies are now in progress to determine the extent to which this fall is

determined by a reduction in muscular contractility of the heart. A precise answer to this question requires the introduction of catheters into the heart to measure pressures. Since these techniques cannot be applied to normal humans, new methods have been tested to estimate the effectiveness of the contraction of the heart by indirect methods, such as by simultaneous recording of the electrocardiogram, phonocardiogram, and carotid pulse. From the time intervals determined from these recordings the effectiveness of the heart beat can be estimated. This method is being applied to normal subjects in the longitudinal study of aging. In subjects with no clinical evidence of heart disease the effectiveness of the heart diminishes on the average up to the age of 60.

Since average values remain stable after this age, or tend to show some improvement, it is hypothesized that individuals fall into two groups, one of which undergoes a gradual deterioration of cardiac function with age and is at increased risk of dying around age 60. Other subjects do not undergo deterioration with age so that they survive into later decades. This hypothesis requires repeated observations on the same subjects as they age. These data will ultimately be available as the longitudinal study progresses. This index may be useful to identify subjects at high risk for the development of heart disease.

### TISSUE CHANGES WITH AGE

Marked changes are found in the mechanical and chemical properties of soft connective tissues, such as skin, with age. Investigators from the Laboratory of Molecular Aging, Gerontology Research Center, have used the healing surgical wound as a model system to examine the mechanisms of this change during the aging process. It was found that young animals produced more collagenous scar tissue than did older animals in repairing incisional wounds. In addition to quantitative differences, there were also qualitative differences in the mechanical behavior of the fibrous tissue deposited. The regenerated tissue in aged animals became significantly stiffer than did that formed in young animals. These results show that the aged animal has less capacity to synthesize a connective tissue network upon the stimulus of a surgical skin incision and that the collagenous tissue which develops assumes the biomechanical characteristics of "older" tissue.

## EYE DISEASES

Progress was made during 1971 in both laboratory and clinic to develop new knowledge about the visual system that may be used to reduce the toll of blindness and visual disability in our Nation.

### DISORDERS OF THE RETINA

Light falling on the retina is the stimulus that creates visual sensations in the brain. The chemical and physiological processes underlying the retina's sensitivity to light are complex and not completely understood.

The retina is a highly organized tissue with an intricate neural network. It requires an adequate flow of blood coursing through its vessels for proper nutrition and function. Interruption of any of the interdependent support systems can result in a disorder which can lead to a partial or total loss of vision. During 1971, the National Eye Institute (NEI) continued its support of research to understand the function of the retina.

The relationship between the layer of cells next to the retina (pigment epithelium) and the light receptor cells, the rods and cones, has been clarified in a number of studies which have a direct bearing on eye disease. The light-sensitive outer segment of the rod cell, which functions in dim light, is composed of hundreds of discs. Dr. Richard W. Young, an NEI grantee at the University of California, Los Angeles, has determined that each retinal rod produces about 100 discs per day in forming its outer segment. The continued production of new discs displaces the previously formed ones, which are pushed gradually towards the pigment epithelium where they are consumed (phagocytized). Thus, the entire rod outer segment is renewed about every 10 days. Older discs are shed and consumed at the rate of 2000 to 4000 per pigment epithelial cell each day. This renewal process does not occur in the cone cells. These findings provide new and important information about the metabolic changes that occur in the normal retina during development and may have an important bearing on the causes of certain hereditary retinal diseases in man.

For instance, the hereditary retinal disorder that occurs in a special strain of rats has been regarded as a model for human retinitis pigmentosa, a progressive usually inherited disorder which causes night blindness and gradual loss of visual field. In NEI-supported studies, Drs. Dean Bok and Michael O. Hall at the UCLA School of Medicine have found that in these rats pigment epithelium cells do not phagocytize debris from the retinal rod cells. This results in the death of these visual cells and blindness. Such a comparison of the normal and pathological changes that can occur in rats with and without retinal dystrophy aids our understanding of human retinal disease.

Dr. Warren L. Herron, Jr. and colleagues at the University of Florida, also working with NEI support, studied this defect in more detail. They found that after the dystrophic rats are 5 weeks old, the production of outer segment material gradually ceases, the rod cells degenerate and the outer segment material is removed by the inner retina. The histologic appearance of the tissue when examined under an electron microscope is similar to that in the end-stage of retinitis pigmentosa in man.

In related NEI-supported research, Dr. T. Wegmann and associates at Harvard University are analyzing the relationships of specific genes to retinal development. They are defining the stages of development particularly vulnerable to the onset of retinitis pigmentosa. This study is also designed to provide a better understanding of the relationship between photoreceptors and the pigment epithelial cells.

The Harvard team use "tetraparental" mice in their study, created by combining two 8-cell stage mouse embryos to form one. The resulting mouse has, in effect, four parents--two from a normal strain and two afflicted with the hereditary retinal disease. In some members of a group of mice produced this way, patches of degenerated tissue were found in the retina interspersed among areas of partially affected or normal tissue. The proportion of normal to abnormal tissue in any individual mouse appears to be directly correlated with the proportion of hemoglobin it has from each of the two strains. This "chimaerism" is believed to be the first demonstrated in mammalian central nervous tissue. The investigators feel that this phenomenon will provide a useful tool for studying neural development and function by permitting the comparison of normal and diseased tissue in the same animal.

## Retinal Detachement Surgery

The neural portion of the retina may become detached from the pigment epithelium due to an accumulation of fluid and other causes. Irreversible damage to the retina will result unless this condition is corrected. Efforts to improve methods for diagnosis and treatment of retinal detachment involve the use of experimental animals in close conjunction with clinical services. NEI-supported studies by Dr. Harvey Lincoff of the New York Hospital-Cornell Medical Center, have shown that the maximal strength of a retinal adhesion at the site of reattachment is obtained in about two weeks. This finding should encourage the ophthalmic surgeon to return his patients to normal activity four weeks sooner than is the current practice after treatment for detached retina, effect a reduction in the cost of medical care, and increase the potential productivity of patients.

A new surgical instrument developed with NEI support by Drs. Robert Machemer and Edward W. D. Norton and colleagues at the University of Miami, simplifies and extends the range of treatment of diseases of the vitreous and complicated forms of retinal detachment. Previous methods for removing vitreous have been clumsy and limited, and involved great risk of damage to the eye. The new instrument, called a vitreous infusion suction cutter, introduced into the eye through a small incision in the sclera, draws the vitreous toward it by suction and simultaneously cuts the vitreous, draws off the cut tissue by suction, and replaces the lost material with a salt solution to restore volume and maintain the shape of the eye.

According to the investigators, the new instrument is of great value in treating vitreous opacities and membranes such as those which form as the result of bleeding in the eye in diabetes, and in treating complicated retinal detachments which result from the vitreous pulling on the retina.

## Light Injury to the Eye

The intensity of light used for the treatment of certain retinal conditions such as edema and detachment, greatly exceeds the

light levels to which photoreceptors are exposed in normal vision. It is essential, therefore, that the capacity of intense light to cause damage to the retina be evaluated.

The widespread use of lasers in medical treatment, research, and industry has increased the potential for accidental injury. Dr. S. Fine and his colleagues at Northwestern University, Massachusetts General Hospital, the Armed Forces Institute of Pathology, and the George Washington University Medical Center are studying the ability of various protective glasses and plastic films to protect the eye against injury form lasers. Their short-term study, supported by an NEI grant, evaluated the degree of protection afforded rabbit eyes by filters of varying optical density. The less dense filters limited eye injury to changes in retinal tissue. The most dense filter tested apparently protected the eye from any laser effects; the least dense one offered some degree of protection and was considered better than no protection at all. Further studies will be required to assess the long-term effects of laser irradiation.

The injurious effects of intense visible light on the eyes are well known and widely discussed every time there is to be an eclipse of the sun. However, several years ago, investigators made the surprising finding that visible light at intensities which are ordinarily encountered can be damaging to the retina of rats. With the need for more knowledge of this phenomenon, several NEI-supported investigators have studied the effects of light on the eyes of experimental animals.

Knowing that continuous exposure to artifical light from incandescent and fluorescent lamps can produce damage to the retina, and that vitamin A deficiency can also lead to retinal damage, Drs. Werner K. Noell and R. Albrecht of the State University of New York at Buffalo studied the relationships between light, vitamin A, and retinal function. They examined the chemistry and physiology of the retina in rats kept in darkness or light, rats exposed to alternate periods of light and dark similar to the variations in day and night, and the effect of the presence or absence of vitamin A in the diet during these periods. The results of these experiments indicate that the normal cyclic variation of light is essential to the health of the retina. In the absence of alternate periods of light and dark, the retina of normal rats can be abnormally sensitized to ordinary levels of light and irreversibly damaged.

## Processing of Visual Information

In NEI-supported studies, Drs. Samuel Sokol and Lorrin A. Riggs at Brown University compared the results of three tests of visual function: psychophysical (based on observation and the patient's own perception), electroretinogram (electrical response of the retina) and visually evoked cortical potentials (electrical response of the brain).

In comparing the three tests they measured temporal resolution--the point at which a flickering light appears steady--under various light frequencies and under different background levels of

illumination and contrast. The electroretinogram measurements indicated a much higher resolution response than the subjects were able to perceive in bright light or than the cortical responses indicated.

These findings point up the selectivity which the brain exercises in handling visual information coming to it from the retina. For proper "perception" of a light impulse by the brain, the stimulus must undergo complex processing before it reaches the brain.

## Photoreceptor Orientation

Dr. Alan Laties of the University of Pennsylvania Medical School and Dr. Jay Enoch of the Washington University School of Medicine, both NEI grantees, have collaborated in determining the direction of arrangement of photoreceptors in the retina. They hypothesized that these light-sensitive elements must either be all arranged perpendicular to the circumference of the eye or be oriented so that they would be parallel to light rays emanating from a point in the front part of the lens. Using histochemical methods, they found that in several species the photoreceptors were arranged to be parallel to entering rays of light. The technical limitations of their histological findings are being checked in further studies. They have also developed the theoretical basis for testing the sensitivity of the living retina to directional rays of light. This will be tested to determine if their experimental results remain valid in a functioning retinal system.

These findings may be important for understanding certain forms of amblyopia in which there is thought to be a disturbance of photoreceptor orientation. This is a condition about which little is known and which such investigations may help elucidate.

## Improved Diagnosis through Basic Research

NEI grantees, Drs. John Dowling of Harvard University and Harris Ripps of New York University studied the adaptation of the skate retina to light and dark by recording signals from specific cells not directly involved with the primary absorption of light. They found that these cells or S-units give electrical signals similar to those seen in a specific portion of the complex electroretinogram. In addition, they found that adaptation to light occurs before electrical signals are emitted from these S-units. These results can now be used as part of a fund of knowledge needed to improve understanding and interpretation of the clinical electroretinogram.

# CATARACT

Studies of the biochemical changes in the lens of a strain of mice with hereditary cararacts were undertaken by Dr. Shuzo Iwata of Harvard University and Dr. Jin H. Kinoshita, formerly of Harvard and now at the National Eye Institute. The afflicted mice appear to grow as well as a closely allied strain of normal mice; the only defect seems to involve the lens. About three weeks after birth,

these mice develop a small pin-head opacity in the lens nucleus. A number of different biochemical properties of these lenses have been studied and compared to normal tissue. The investigators first found that just prior to the appearance of the pin-head opacity, the only significant changes were increased uptake of sodium and water. Then, using radioisotopes, earlier changes were detected. Eleven days after birth, when sodium and water levels are still normal, a marked loss in the ability of the lens to concentrate rubidium was observed. In addition, in this very early stage, the activity of a specific enzyme system in the lens (the adenosine triphosphate system which requires sodium and potassium) was operating at only half its normal rate. These results suggest that the primary defect in these lenses is in the transport of these substances.

In other studies, Dr. Kinoshita and R. J. A. Jedziniak of Harvard added to earlier evidence that the enzyme aldose reductase plays a primary role in the initiation of sugar cataracts, those which blind babies suffering from galactosemia and which sometimes occur in diabetes patients. In their search for inhibitors of the enzyme, which could prevent or delay the development of cataracts, the investigators succeeded in purifying the enzyme and studying its properties. They found it to consist of two smaller units, each inactive by itself. Aldose reductase inhibitors, such as tetramethylene glutaric acid (TMG) thus inactivate the enzyme by splitting it in two.

The investigators have now identified another inhibitor, triphosphopyridine nucleotide, which is active in much lesser amounts than TMG. This compound is found in the normal lens at a level sufficient to block cataract formation. These findings have enabled the investigators to deduce the general organic structure required for aldose reductase inhibitors, and may lead to the development of other such compounds.

## Cataract Surgery

Dr. Stephen Fricker of the Massachusetts Eye and Ear Infirmary, Boston, has developed a method for improving the sensitivity of the test to measure visual evoked response, the electrical activity in the brain which occurs as a result of a visual stimulus.

Dr. Fricker's system greatly refines the recording of the signal as a function of time, a development which makes it possible to determine whether or not the retina is normal, even if a cataract is present. This information could be crucial in deciding whether or not it is worthwile to remove a cataract in a particular patient.

## INFLAMMATORY DISEASE

Herpes simplex infection is one of the most serious and important causes of corneal disease. Drs. Herbert Kaufman and Rakesh Goorha of the University of Florida hve been searching for agents useful for treating this condition. One of the agents they have evaluated is interferon, a group of antiviral proteins which inhibits the reproduction of viruses by acting on the host cells.

It is effective against most viruses and is non-toxic to the host.

In NEI-supported studies, the investigators demonstrated that the owl monkey can be protected from herpes virus infection by treatment with human leukocyte (white blood cell) interferon. This protection is not complete; some of the eyes do develop ulcers, but they are not as severe as those which occur in unprotected animals. Additional studies are in progress to determine the best treatment regimens and the length of time for which the protection is effective. This finding encourages the investigators to study the practicality of similar administration to humans for the prevention of recurrent herpes infection of the cornea.

## Corneal Disease

At Boston University Medical Center, Drs. Howard M. Leibowitz and Perry Rosenthal conducted an NEI-supported study of the use of hydrophilic (soft) contact lenses in the treatment of corneal disease. Patients, having corneal ulcers (usually caused by herpes simplex virus), which had not responded to conventional treatment, were fitted with a hydrophilic contact lens. The outer layer of the cornea was removed prior to lens fitting, and the lens remained in place for one week. For all cases in this series there was complete relief from pain, and low rate of recurrence. Although the mechanism involved in this success is not definitely known, the investigators believe that the soft lens acts as a protective bandage shielding the ulcer from irritating contact with the lids as well as from environmental irritants. The soft lens may also provide a structural framework upon which the regenerating corneal epithelium can grow. The same investigators went on to show that soft contact lenses can relieve the pain and accompanying symptoms associated with blistering erosion of the cornea (bullous keratopathy). The lens was well tolerated and may be worn for weeks without adverse effects. They also found that the use of sodium chloride drops in connection with the soft contact lens resulted in significant improvement in visual acuity in approximately half their patients with early bullous keratopathy.

## Corneal Edema

The transparency of the cornea is dependent on the precise regulation of its water content. In several diseases, and after surgery on the eye, the cornea may become swelled with water (edematous). Working under an NEI grant, Drs. M. N. Luxenberg and K. Green of Johns Hopkins University evaluated various commercially available substances for their ability to reduce the thickness of a swelled cornea. These investigators found that a simple ointment of 5 percent sodium chloride was the most effective agent for returning the swelled cornea to normal and permitting the recovery of vision.

## Sympathetic Ophthalmia

Evidence that may explain why severe injury to one eye will, in some persons, cause a serious inflammation to develop in the other eye was presented by Dr. Vernon G. Wong and associates of NEI.

The condition, called sympathetic ophthalmia, because of the "sympathetic" involvement of the uninjured eye, has long puzzled physicians and scientists. In recent years, autoimmunity, the body's allergic reaction to its own tissue, has been suggested as a cause. Dr. Wong's investigations suggest that in sympathetic ophthalmia the initial injury may "sensitize" the body's white blood cells (lymphocytes), causing them to attack the other eye as if it were foreign tissue.

This finding indicates that autoimmunity plays an important role in sympathetic ophthalmia, although it may be the sole cause of this condition.

## GLAUCOMA

Patients with glaucoma are being treated and followed at a number of clinics whose work is supported by the National Eye Institute. In addition, basic research is being pursued to follow up several aspects of this disease. Glaucoma induced by steroid drugs has been of particular interest, both as a test for susceptibility to glaucoma and as a potential cause of blindness.

### Inhibiting Steroid Response

Steroids are among the most effective drugs known to medicine for treating inflammatory disease. Yet they must be used cautiously in large doses and for prolonged periods because in some people they cause a rise in intraocular pressure which may lead to glaucoma and blindness. Dr. Raymond P. Le Blanc and colleagues at the Washington University School of Medicine reported some success using inhibitors of protein synthesis in preventing a steroid-induced increase in intraocular pressure.

### Relationship with Diabetic Retinopathy

Dr. Bernard Becker and his associates at the same institution have found a relationship between abnormal results from the glucose tolerance test used to detect diabetes and a positive pressure response to steroids. These findings suggest that there is an association between diabetes and open-angle glaucoma. Dr. Becker therefore suggests that doctors check diabetics for glaucoma.

Conversely, in patients with glaucoma or high response to steroids, Dr. Becker recommends checking for diabetes. Also, he notes, steroid-responders with a positive glucose tolerance test appear to be more susceptible to visual field loss than those with a negative glucose tolerance test.

Finally, positive glucose tolerance tests are more prevalent in glaucoma patients with lower intraocular pressures. Patients with positive test results appear to lose visual field at lower intraocular pressures than do those with negative test results. Thus, patients with low pressure glaucoma may be more inclined to diabetes; glaucoma patients with positive glucose tolerance tests may require more rigid control.

## Steroids for Eye Disease

Dr. Steven M. Podos, also of Washington University, and his colleagues are conducting clinical studies to find topical steroids which can be used to treat inflammation of the eye without causing glaucomatous pressure elevations. They, along with other NEI grantees, have reported the differing effects of several steroids on the intraocular pressure and have identified one drug, medrysone, which appears to be relatively free of this side effect.

## Secondary Glaucoma

Patients with uveitis may develop secondary glaucoma. Drs. W.M. Townsend and Herbert E. Kaufman of the University of Florida have studied inflammation of the cornea caused by herpes virus and accompanying uveitis in rabbits. They found that many of these animals developed glaucoma. While the treatment of uveitis cured the glaucoma in some cases, in others the glaucoma persisted and damage to the cornea occurred. These studies may be valuable in assessing the risk and the need for early therapy of secondary glaucoma in uveitis patients.

## Circulatory Studies

Understanding the effect of intraocular pressure on the blood circulation of the eye is of great importance to knowledge of normal physiology and the development of glaucoma. Dr. Mansour Armaly and his associates at George Washington University have studied the effect of intraocular pressure on the blood circulation of the choroid, the vascular layer underlying the retina, in cats and rhesus monkeys. They have shown that the temperature of the retinal vessels varies with changes in intraocular pressure and systemic blood pressure. These temperature changes reflect alteration in the volume of blood flow. These findings suggest that circulation in the choroid responds to an increase in intraocular pressure. Circulation in the optic nerve was also found to be very sensitive to intraocular pressure variations, particularly that portion outside the eye. Studies are in progress to understand more clearly how this effect occurs, its implication for the development of glaucoma, and its response to drugs and denervation procedures.

## Studies of Aqueous Flow

NEI Director Dr. Carl Kupfer reported that in fetal eyes the ability of aqueous fluid to flow out of the eye develops at the time when openings form in the cellular layer which covers the trabecular meshwork. The meshwork consists of fine outflow channels which drain the fluid from the eye. Dr. Kupfer raised the question of whether the cause of some cases of congenital glaucoma might be the failure of these openings to develop. If further research bears this out, improved surgical correction of such a defect may be possible.

Useful information on the various aspects of aqueous flow in glaucoma must be based on detailed knowledge of such dynamics in normal individuals. Dr. Kupfer and his associates have made the

first detailed measurements of intraocular pressure, flow of fluid into and out of the eye, and pressure in the veins in the conjunctiva in normal subjects. These measurements were the same in each individual after repeated testing and there was good agreement between the values obtained for the right and left eye, making possible evaluation of the effects of drugs on one eye while the other serves as a control. Such studies have shown the different ways in which various drugs affect the intraocular pressure, both alone and in combination. These findings are expected to have significant impact on the development of more exact methods for treating glaucoma and on evaluating the effects of new drugs as they are developed.

## EYE MUSCLE DISORDERS

Studying the control of eye movements, Drs. Alexander A. Skavenski and Robert M. Steinman at the University of Maryland have shown that a subject in complete darkness is able to maintain control of his line of sight to within two degrees for up to two minutes. This NEI-supported investigation demonstrates the existence of some system for controlling eye position which is not dependent on an image for fixation.

Such information on the control of eye position is important for the diagnosis and treatment of a number of conditions which affect the oculomotor system, such as multiple sclerosis, or in conditions of muscle imbalance such as strabismus in children.

### Strabismic Amblyopia

Better understanding of strabismic amblyopia has been gained by the studies of Drs. H. W. Perry and D. G. Childers at the University of Florida. Amblyopia is defective vision without obvious pathologic cause. It often results as a consequence of strabismus, in which one eye deviates inward or outward. Strabismic amblyopia is usually considered to be caused by suppression of the false image arising from the deviating eye. The Florida investigators have devised techniques for quantitatively measuring the poorly understood concepts of dominance and supression in normal and amblyopic children. The results of their experiments clearly show that some "normal" children approach those with amblyopia in the extent to which they suppress vision in one eye or the other. Therefore the basic processes underlying both normal binocular vision and amblyopic vision differ only in degree and not in kind. These findings may have an important bearing on continuing research in strabismic amblyopia.

## PHARMACOLOGY OF THE EYE

A broad program on the action of various toxic substances on the eye is in its seventh year of NIH grant support at the University of Chicago under the direction of Dr. Albert M. Potts. Recently, Dr. Potts and Dr. Pin Chit Au reported their studies on the storage of thallium in ocular structures. Thallium, a metallic ion found in rat poison, is a cause of human poisoning with serious effects on the

eye. The investigators found that thallium is stored in the eye by at least two mechanisms: one, involving binding by the melanin pigment, resulting in exceptionally high levels of the metal in the pigmented tissues (iris, choroid, and ciliary body), the other involving substitution of thallium for potassium in the normal metabolism of the lens and possibly the retina and optic nerve. The second mechanism could account for the acute inflammation of the optic nerve which is the usual ocular symptom of thallium poisoning.

### Role of Dopamine

Dr. Steven G. Kramer, also of the University of Chicago, investigated the role of the biologically important compound dopamine in the visual system of cats. Previous studies had identified a layer of dopamine-containing fibers and cell bodies at the junction of two retinal layers. Dr. Kramer found that more dopamine is released by light-adapted retinas than by dark-adapted ones. It is known that the drug reserpine depletes nerve tissues of dopamine and that animals treated with this drug become abnormally sensitive to light. Dr. Kramer's new findings indicate that dopamine is involved in regulating the light sensitivity of the retina and lead to the speculation that pharmacological control of retinal sensitivity may be feasible.

## ENVIRONMENTAL HEALTH SCIENCES

### CHLORINATED HYDROCARBONS

Interest in exploring the nature and hazardous properties of many chlorinated hydrocarbon compounds is based on the persistence and consequent accumulation of these compounds in man as well as in his environment. Important gains were made during the year in adding to existing knowledge.

### Polychlorinated Biphenyls

In December 1971 the National Institute of Environmental Health Sciences (NIEHS) sponsored an international conference to assess the state of knowledge of the polychlorinated biphenyls (PCB's). This conference grew out of a recommendation by an Interdepartmental Task Force in the fall of 1971 that Government resources be devoted to defining and dealing with this problem. Emphasis at the conference was on whether these industrial chemicals can affect human health. The chemistry of the PCB's, how they are disseminated in the environment, instances of contamination and the harmful result in wildlife and humans, as well as possible compounds which might serve as alternatives, were discussed.

The PCB's have been used for more than 40 years. They are heat-resistant liquids and as a result have a wide range of industrial applications. They have been used as electrical insulating fluids for capacitors and transformers, industrial fluids for hydraulic, gas-turbine, and vacuum pumps, and plasticizers.

An episode of acute poisoning affected 1000 people in Japan and resulted in cases of liver damage, miscarriages, stillbirths, and transplacental effects resulting in abnormal pigmentation from "rice-oil" disease (Yusho). This was the result of accidental contamination of cooking oil by PCB's. Recent episodes of accidental contamination of poultry and milk from leaking of PCB heat transfer fluids and from paints containing PCB's for silo treatment have focused on the need for additional studies, to assess the risks, if any, involved in subacute and chronic exposure to the PCB's.

Drs. W. E. Wilson and C. Sharp of NIEHS are studying aspects of the interaction of a beef brain enzyme with PCB's. This enzyme is necessary for neuronal function and proper salt and water balance. Enzyme activity was inhibited 50 percent by the presence of PCB's at concentrations of 8-12 parts per million (ppm), comparable to similar inhibition of the enzyme by 9 ppm of DDT.

The intestianl absorption of a number of these compounds in the rat has been studied by Drs. P. W. Albro and L. Fishbein of NIEHS. PCB's having from 1 to 6 chlorine atoms per molecule were very well absorbed when fed in single doses between 5 and 100 milligrams per kilogram of body weight. Less than 10 percent of the amount fed was excreted.

## Dioxin

Previous studies by NIEHS scientists showed that the earlier commercial grade herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) which contained 30 ppm of the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was a teratogenic and fetotoxic agent for mice and rats. Teratogenic effects (physical defects produced in the developing embryo) in mice were manifested by cleft palate and cystic kidney; fetotoxic effects in rat fetuses were manifested by bleeding in the gastrointestinal tract and death of kidney cells. Subsequently studies were undertaken to establish whether 2,4,5-T or TCDD was responsible.

Both the herbicides were found to be teratogenic in all three strains of mice used. Additional studies on the dioxin TCDD have been started by Dr. John Moore of NIEHS to identify the sequence of toxicological processes following exposure to TCDD and to determine whether any abnormalities are detactable postnatally.

In the first study rats that received a daily dose of 10 micrograms of TCDD per kilogram of body weight for 10 days showed a cessation of weight gain and a subsequent weight loss. The TCDD dose administered to mice was progressively reduced in an attempt to find a "no-effect" level. To date doses have been decreased to 0.1 microgram per kilogram of body weight and administered for 1 day only. Kidney anomalies were still observed in the offspring. Studies on TCDD will be continued in order to clarify the mechanism by which it produces its toxic effect and to compare the prenatal and postnatal effects of the dioxin exposure.

The environmental fate of the dioxins has been a matter of

concern because of extensive use of chlorinated phenols in which the dioxins may be found as contaminants. Decomposition by sunlight represents one potential route of elimination from the environment. Accordingly, NIEHS grantees Drs. D. G. Crosby and A. S. Wong of the University of California at Davis in cooperation with Drs. J. R. Plimmer and E. A Woolson of the Agricultural Research Service in Beltsville, Md., have investigated the rates and products of this photodecomposition under a variety of conditions.

These grantees found that the toxic TCDD and related compounds, or compounds descended from TCDD, decomposed rapidly in alcohol under artificial and natural sunlight, the rate of decomposition depending upon the degree of chlorination. These experiments indicate that light in the solar radiation at the earth's surface can accomplish the rapid destruction of the dioxin in the presence of organic hydrogen-donors. Such donors could be represented environmentally by the waxy cuticles of green leaves, surface slicks on water or the spray oil or aromatic solvent so often incorporated into pesticide formulations. These experiments also revealed that photodecomposition of the dioxin was negligible in acqueous suspensions and on wet or dry soils.

## Mirex

The suggested use of the insecticide Mirex to control the spread of the imported fire ant over the entire Southeastern United States has recently been subjected to increasing scrutiny. The report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health, 1969, classified Mirex as an agent capable of producing tumors. Because Mirex is applied in a bait which eventually decomposes, releasing the insecticide into the soil and run-off water, its degradation by soil and water-borne microorganisms would play an important role in the elimination of this insecticide from the environment. If Mirex cannot be degraded by such microorganisms, its further use could result in possible dangerous accumulations.

Dr. Felton Hastings of the U. S. Forest Service, by agreement with NIEHS, is studying the microbial degradation of Mirex. Studies on the effect on soil microorganisms indicate that Mirex at the concentrations used has no effect on populations of these microorganisms. Studies on the metabolism of Mirex by soil microorganisms indicate so far that Mirex has not been degraded by them.

Dr. P. W. Albro of NIEHS has found Mirex in samples of catfish and oysters taken from areas in which Mirex could be anticipated to be present. Dr. H. B. Matthews of NIEHS is studying the metabolism of Mirex in rats. Low doses (up to 90 ppm) of Mirex fed in the diet did not produce any damage to tissues. Mirex administered to rats by intubation at doses of 10-25 milligrams per kilogram of body weight per day for up to 6 days, however, showed very definite tissue damage. The highest concentration of Mirex was in the gastrointestinal tract.

## DDT

Despite a quarter century of research on the biological effects of DDT, interest in its effects continues. Some of its most significant effects and mechanisms of action, such as its tumor-causing properties, have been discovered only recently.

It has long been known that DDT is a neurotoxin, a substance that is poisonous or destructive to nerve tissue. However, most studies of the effects of DDT on the mammalian nervous system were performed before the advent of modern neurophysiological techniques and equipment; thus it is important to re-evaluate the neural effects of DDT especially in intact animals. Dr. Dorothy E. Woolley, an NIEHS grantee with the University of California at Davis, has investigated the effects of DDT on brain electrical activity in rats.

After a single lethal dose of DDT was administered to rats bearing chronically implanted brain electrodes, changes in spontaneous and evoked brain electrical activity could be detected before gross changes in behavior such as tremors occurred. The greatest increases in spontaneous and evoked electrical activity occurred in the cerebellum (the part of the brain concerned with the coordination of muscles and maintenance of bodily equilibrium) thus supporting earlier suggestions that the cerebellum is a primary target for DDT. Marked increases in the arousal waves in brain electrical activity was in agreement with behavioral hyperexcitability. High doses of DDT resulted in respiratory failure whereas low doses have been shown to stimulate respiration.

## ORGANOPHOSPHORUS PESTICIDES

Because of the attempts at replacing residue-leaving chlorinated hydrocarbon insecticides by effective but less permanent organophosphorus insecticides, emphasis has focused on toxicological effects after chronic exposure to these chemicals.

### Malathion

Organophosphorus pesticides may inhibit transmission of an impulse from one nerve fiber to another across a synaptic junction. Several organophosphorus pesticides are also capable of producing physical defects in the developing embryo (teratogenic effects) especially in birds, but the mechanisms responsible have not yet been discovered.

Malathion, a widely used insecticide, is teratogenic to the chick embryo although safe for use around adult chickens. It has been shown that malathion must be converted to a related chemical, malaoxon, in order to inhibit cholinesterase.

In work undertaken by Dr. N. E. Walker, and NIEHS grantee at the University of California at Davis, the abnormalities which resulted from exposure of chick embryos to malathion or malaoxon, although similar, differed in some ways from those expected if malathion can act only after conversion to malaoxon. The discrepancies led to a

study of the two compounds on embryonic development. It was found that malathion when applied to chick embryos by incorporation into yolk was more teratogenic than malaoxon. This indicates that malathion itself is biologically active.

## INDUSTRIAL CHEMICALS

The biological effects of nitrilotriacetic acid (NTS) are of great current interest because of the potential use of this compound as an ingredient in household detergents. In study supported by NIEHS conducted by Dr. Robert A. Goyer and co-workers at the University of North Carolina, varying doses of sodium NTA in the diet alone and in combination with a standard amount of lead in drinking water were fed to rats. Rats on the highest dose of NTA with or without lead acetate became moribund and survivors were sacrificed after 4 weeks. These rats had glucose in the urine. Rats receiving lesser doses of sodium NTA with or without lead were sacrificed after 10 weeks. Brain, liver, pancreas, and kidney appeared normal. The lead content of tissues was not elevated in rats receiving sodium NTA plus lead.

All rats had some degree of hyperglycemia, an abnormal sugar content in the blood. Further studies regarding the mechanism of NTA-induced hyperglycemia are in progress.

### Phthalate

Phthalate is a substance used as an additive in plastics to make them flexible and has wide use in such materials as food wraps, bags, and blood bags. Dr. Robert Rubin, an NIEHS grantee at Johns Hopkins University, has determined that phthalate esters can be leached from the containers, and food or biological materials stored for long periods of time in flexible plastic containers may be contaminated.

Approximately a billion pounds of phthalates are produced annually. Phthalate esters have been identified in crude oil and in water as well as in fish at concentrations of 10 ppm. In addition phthalate esters have been found in fish meal and cotton seed meal. While phthalate esters have a very high non-effect level when given orally to several species, nothing is currently known of the effects of continued inhalation at low levels, such as from new car interiors and plastic furniture.

## HEAVY METALS

Contamination of the environment with heavy metals has recently been recognized to be a serious problem. Mercury is a particularly hazardous element not only because of its high, broad-spectrum toxicity, but also because elemental mercury and inorganic mercurials are converted into alkyl mercury by microorganisms and can cycle indefinitely in the food chain. Recent studies have revealed higher than allowable levels of methyl mercury (an alkyl mercurial) in some fish, fowl, game, foodstuffs, and water.

## Mercury Research

Since the 1940's many investigators have described the toxicology of alkyl mercury compounds with reference to contamination of Minamata Bay, Japan. Residents there have been afflicted by a severe disorder resulting from poisoning by alkyl mercury, leading to severe permanent neurological and mental disabilities or death. This disorder has come to be known as Minamata disease. Recent findings of high levels of methyl mercury elsewhere in the environment have raised new questions concerning its genetic and teratological effects, the site and mode of its action, and its combined effects with other compounds.

NIEHS scientists Dr. Ronald Klein, Dr. Sheldon Herman, Dr. Paul Brubaker, and Dr. George Lucier, in collaboration with Dr. Martin Krigman of the Department of Pathology, University of North Carolina School of Medicine, were able to develop a highly reproducible model of acute alkyl mercury intoxication (Minamata disease) in adult rats following daily subcutaneous injections of methyl mercury hydroxide for 7 days. The dosage produced weight loss followed by decreased activity, failure of muscular coordination, weakness, and the hind-limb crossing phenomena. This model of acute Minamata disease in the rat should be extremely useful to other investigators who plan to study the pathogenesis and treatment of methyl mercury intoxication.

## Antidote for Mercury

Two NIGMS grantees at the University of Rochester have discovered a very promising antidote for mercury poisoning. The research by Drs. Louis Lasagna and Thomas W. Clarkson is based upon the observation that methyl mercury ingested in food is almost completely absorbed from the stomach into the blood stream, where it moves rapidly to the liver and thence is excreted back to the large intestine or gut through the bile. This suggested to the scientists that an absorbent material might be taken by mouth to trap the mercury while it is cycling through the intestine, and that it might therefore be excreted more rapidly in the feces. They subsequently were able to demonstrate that such an absorbent material, a resin substance sepecially synthesized for this investigation by the Dow Chemical Company, is indeed an effective mercury trapping agent. Mice given injections of methyl mercury and fed the resin in their diet excreted the mercury three times faster than did control animals. If the resin is similarly effective in man it would be an important treatment for methyl mercury poisoning.

## Lead and Cadmium

The clinical manifestations of many overt heavy metal intoxications are well documented. There is much to be learned, however, concerning the subclinical toxicity of metals. In the past year, NIEHS has awarded a program grant at the University of North Carolina at Chapel Hill to investigate the biochemical correlates of molecular and subcellular disruption due to heavy metals. Lead and cadmium are the principal elements under study and their effects are being observed in the renal system, the nervous system, and the reproductive system. The investigators are working initially at

high metal concentrations to determine the toxic mechanisms of lead and cadmium and to discover possible joint action of these and other agents in which the combined effects are greater than the sum of their individual effects. Long-term, low-level exposure to these and other heavy metals will be studied later.

## PSYCHOACTIVE FLAVORING AGENTS

Allylic and propenylic benzene derivatives are representative of a broad class of agents that are widely found as components of essential oils and flavoring agents. One of these, myristicin, has been identified in nutmeg, parsley, carrots, bananas, black pepper, and processed tobacco. Another, safrole, is a constituent of the essential oils, sassafras, star anise, and camphor, and a minor constituent of oils of nutmeg, mace, cinnamon and wild ginger. It has been widely used until recently as a flavoring agent in root beer, chewing gums, toothpastes, and pharmaceuticals and to scent soaps and cosmetics.

Dr. E. O. Oswald of NIEHS has been studying the mammalian metabolism of myristicin and safrole. It has previously been postulated that biological conversion to amphetamines was a requirement to explain the observed psychoactive properties of myristicin, for example. No amphetamine-type compounds were detected in Dr. Oswald's studies; however, nitrogen-containing metabolites from myristicin and safrole were found and identified. This finding may contribute to the better understanding of the physiological as well as pathological action of these substances in natural food products and essential oils.

## RADIATION HAZARDS

Although substantial amounts of research have been conducted on the biological effects of laser radiation, there remains a need for more detail on laser interactions at the molecular level. NIEHS supports a research project at North Eastern University at Boston concerned with specific photochemical effects of ruby laser radiation that might lead to biological damage. Tests have been conducted on relatively simple compounds to provide a basis for more complicated studies on biologically significant molecules such as amino acids, proteins, and DNA.

## MUTAGENS

Because of their potential for altering the genetic material of living persons so as to damage future generations, mutagens represent potentially the most serious of the environment hazards. Unfortunately, the test systems for evaluation of mutagenic hazards for man have been inadequate.

Studies were initiated this year by three NIEHS researchers, Dr. W. Gary Flamm, Dr. Donald Clive, and Michael Machesko, to the development of sophisticated techniques for determining genetic mutations in the laboratory. Some chemicals have been shown to exhibit species-specificity in their mutagenic activity. This has

been attributed to differences in host metabolism of the compound (converting non-mutagens to mutagens or vice versa) or to differences in chromosomal architecture between, for example, bacteria and mammals. The ideal mutagen assay system should combine the operational ease and high sensitivity of bacterial test systems with the biological relevance of expensive and time-consuming whole mammal test systems. This system should, in effect, expose a large number of mammalian genomes (the complete set of hereditary factors) to the test compound and its products of mammalian metabolism in a mammalian milieu.

The intent of these researchers was to use mouse lymphoma cells as the mammalian genetic indicator implanted within a mouse abdominal cavity to obtain the mammalian metabolism and milieu. Experiments were undertaken to test the feasibility of confinement of the cells in dialysis bags, which permit diffusion of the mutagen and its metabolites implanted in the peritoneal cavity. Work to date suggests that this mouse-mouse host-mediated assay for chemical mutagens combines operational convenience and high sensitivity with biological relevance to man.

## Hycanthone

The genetic test system developed by Drs. Flamm and Clive has already been utilized in investigating hycanthone, a new drug which has been found promising for the treatment of schistosomiasis, a disease resulting from infection with Schistosoma flukes (S.mansoni and S. haematobium).

Hycanthone methanesulfonate has been administered to approximately 100,000 people in Africa and Brazil in extensive field trials. Its efficacy, low toxicity at therapeutic dosages, and ease of administration have generated considerable enthusiasm for its use and its potential value in expanded anti-schistosomal campaigns. The drug's long-term efficacy, however, has been challenged. Some researchers have demonstrated the development of hycanthone-resistant S. mansoni 6 to 12 months after treatment of rodents infected with the schistosome. Others have demonstrated that hycanthone is a potent mutagen in Salmonella, a bacterium.

In studies to determine whether hycanthone is mutagenic to mammalian cells, the NIEHS researchers found that cultures treated with hycanthone methanesulfonate exhibited a substantial increase in mutant frequency roughly proportional to the concentration of the compound. They now will attempt to determine whether hycanthone will revert its own induced mutants as well as those induced by well-characterized mutagens. In this way it is hoped to learn whether hycanthone is essentially a mutagen for mammalian cells as it is in bacterial systems. Other scientists at the NIEHS are studying the teratogenic potential of this drug and have thus far obtained positive results.